DISSERTATION ON

COMPARISON OF MODIFIED FLUORESCENT METHOD AND CONVENTIONAL ZIEHL NEELSEN METHOD IN THE DETECTION OF ACID FAST BACILLI IN LYMPH NODE ASPIRATES.

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Under the guidance of

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CERTIFICATE

This is to certify that this dissertation titled **"COMPARISON OF MODIFIED FLUORESCENT METHOD AND CONVENTIONAL ZIEHL NEELSEN METHOD IN THE DETECTION OF ACID FAST BACILLI IN LYMPH NODE ASPIRATES"** is the original and bonafide work done by **Dr.T.UMASANKAR** under the guidance of Dr. P. Arunalatha, M.D., Professor, Department of Pathology at the Government Stanley Medical College & Hospital, Chennai – 600 001, during the tenure of his course in M.D. Pathology from May-2009 to April-2012 held under the regulation of the Tamilnadu Dr. M.G.R. Medical University, Guindy, Chennai - 600032.

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CONTENTS

ABBREVIATIONS

AFB	-	Acid Fast Bacilli
AO	-	Auramine O
FNAC	-	Fine Needle Aspiration Cytology
H&E	-	Hematoxyllin & Eosin
MTB	-	Mycobacterium Tuberculosis
ZN	-	Ziehl Neelsen

INTRODUCTION

INTRODUCTION

Pulmonary Tuberculosis (TB) is a contagious bacterial infection that involves the lungs, but may spread to other organs. The great pioneer of bacteriology**, Robert Koch,** discovered a bacterium which he called bacillus tuberculosis and is now known as Mycobacterium tuberculosis, is the etiological agent of tuberculosis[1]. Tuberculosis continues to be a major health problem in developing countries due to poor sanitation, overcrowding and lack of knowledge about the disease. AIDS patients show coinfection with Mycobacterium tuberculosis. More than 50% of Tuberculosis cases are from Asia and Africa alone. It has been estimated that one patient die every minute due to MTB in India. Symptoms of pulmonary TB include cough , coughing up blood(hemoptysis), excessive sweating especially at nights, fatigue, evening rise of temperature, unexplained weight loss. Other symptoms that may occur with this disease are dyspnea, chest pain, wheezing., clubbing of the fingers or toes (in people with advanced disease), enlarged or tender lymph nodes in the neck or other areas, pleural effusion, unusual breath sounds (crackles)[2,3].

TB is a preventable and potentially curable disease provided it is detected earlier in the course of the disease. Microscopy is the most common case detection test in use. Microscopy is inexpensive, relatively rapid to perform, and, in countries where tuberculosis is endemic, is highly specific.

The average sensitivity of sputum microscopy for pulmonary tuberculosis in immunocompetent individuals is less than 60% compared with culture, even in research settings[5]. Microscopy can also be done in other pathological materials like lymph node aspirates and body fluids including CSF.

The diagnostic value of FNAC of lymphnodes in tuberculous lymphadenitis has been emphasized by several workers[6,7,8]. FNAC examination is simple, and a relatively painless and less cumbersome procedure introduced first by Martin[9], can be adopted in lieu of biopsy. This can be carried out as an O.P.procedure and preparation of the smears can be carried out in the laboratories even at the peripheral hospitals. This will serve as an effective adjuvant in arriving at an appropriate diagnosis.

Identification of AFB in lymph node aspirates by the routine H&E stain is difficult. We give a diagnosis of granulomatous lesion based on the presence of epitheloid cell,giant cells and caseous necrosis.As the etiology of granulomatous lesions are varied we need to arrive at a definitive diagnosis for the cause of the granulomatous lesions MTB is the commonest cause of granulomatous lesion in our country.In order to give a definitive cytological report that the cause of granulomatous lesion is due to MTB,we have to employ special stains like Ziehl Neelsen .Ziehl Neelsen method is the current gold standard test employed for the identification of AFB on FNAC smears. But the sensitivity is low .MTB culture though sensitive and specific takes a

longer time for the growth of the organism. The tests based on PCR have shown promise for the detection of mycobacteria in clinical samples[15]. However, several different PCR systems that have been described for the diagnosis of tuberculosis have produced widely differing results with regard to the sensitivity of the assay with different types of clinical samples .PCR although a sensitive test detects both viable and non viable bacilli[16]. Peripheral blood appears to be the clinical material of choice for PCR, especially in cases of disseminated and extrapulmonary forms of the disease[17]. Hence we need a test which should be sensitve, rapid, easy to perform and cost effective as well.

Auramine O has been used in fluorescence acid-fast microscopy since it was first introduced by Hageman in 1938 and reported by Richards et al[11]. This method is said to be more sensitve than the Ziehl Neelsen method and can be performed on lymph node aspirates as well.

In this study the sensitivity, specificity, efficacy and other advantages of using AO stain over the conventional gold standard Ziehl Neelson stain in granulomatous lesions of lymph node are compared and the results analysed.

AIMS AND

OBJECTIVES

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1. To correlate the modified fluorescent method with the conventional ZN method, and also to compare the results with routine cytology on lymph node aspirates reported as granulomatous lesions for the detection of AFB.

2. To provide a definitive cytological diagnosis that the cause of granulomatous lesion is due to AFB.

3. To study the efficacy and advantages of using Auramine O stain under fluorescence microscope.

REVIEW OF

LITERATURE

REVIEW OF LITERATURE

Bhatia VN, et al, compared Auramine staining with Ziehl-Neelsen staining of Mycobacterium leprae in skin smear slide. The auramine method was found to be more sensitive than Ziehl-Neelsen method and is useful in detecting small number of Mycobacterium leprae in skin smears. The inter-observer variance was found to be minimal with auramine staining[18].

Myrna T. Mendoza, M.D. and Cristino P. Narciso, R.M.T et. al., found that use of fluorescent acid fast staining with sputum microscopy[FAM] gives better overall accuracy.Out of the total 2183 specimens 159 smears were positive for acid-fast bacilli by the FAM and 132 were positive by Z-NT bright field microscopy. By either method the positive yield from the total specimens was 159 or 7.0%. Both techniques were in agreement that 2024 (92.7%) were negative smears. The total positive yield from the 2183 specimens was slightly higher by FAM 159 (7.0%) as against 132 (6.0%) positive by the Z-NT method. Higher grades of positive smears were noted by the FAM technique. 24 more positive AFB smears (1.09%) were picked up by FAM which were completely missed by the Z-NT method. Higher positive yield by FAM was noted which suggest greater sensitivity of the technique. Higher grades of positivity were also observed with the FAM, similar to the H.L. David study cited by Toman. Expectedly, due to the lower magnification used to scan fluorochrome stained smears, the microscopist was

able to view a much larger area of the smear. In addition, fluorescing yellow orange bacillus in a dark background is easier for the eye to detect. These findings showed that it is the more reliable technique for AFB detection especially in laboratories with a heavy work load for sputum microscopy[19].

F. Ba, H. L. Rieder et. al., compared fluorescence and bright-field microscopy for acid-fast bacilli. Two smears from 2630 consecutive sputum specimens between January 1996 and June 1998 were prepared for examination of one smear each by the Ziehl-Neelsen technique and fluorescence microscopy at 1000x magnification. The time required to screen and declare a slide as negative was determined for both techniques in a sample of 68 slides. Concordance was 96.9% and 92.3% for diagnostic and follow-up examinations, respectively. The result was similar with both techniques for specimens with at least 10 bacilli per 100 fields, but higher with fluorescence microscopy in those with fewer than 10 bacilli per 100 fields. The mean time required by fluorescence microscopy before declaring a slide as negative with the same magnification was 3 minutes 34 seconds, compared to 7 minutes 44 seconds with the Ziehl-Neelsen technique. The results obtained with one technique are highly reproducible by the other. Fluorescence microscopy appears to be more likely to detect bacilli in paucibacillary cases than bright-field microscopy, and it more than halves the required examination time[20]**.**

B.W. Oromcan et. al., designed a study to compare the performance of Ziehl Neelsen(ZN) staining and Auramine fluorescent microscopy staining techniques in detecting the presence of Mycobacterium tuberculosis in sputum. This was a cross sectional study design conducted at Mengo hospital laboratory that is located in Rubaga division Kampala district. All those patients whose smear results by routine ZN stain were reported as negative were selected for the study. 124 sputum samples were studied which included 122 sputum samples that were negative for AFB by routine ZN smear technique and 2 sputum samples which were of low positivity that is + AFBs seen with routine ZN. Four slides were labeled for each sample as ZN, auramine-o preparation (AP), The finding of this study revealed that 0.8% that was negative for ZN was positive when auramine-O technique was performed. This study also found that 1.6% negative cases when ZN concentrates were used was positive with Auramine-O concentrates preparation. Also 4.0% positive cases when ZN concentrates were used where negative with Auramine-O concentrates preparation. 3.2% of positive cases when Auramine concentrates were used were negative with ZN concentrates preparation. 0.8% negative cases with auramine-O were positive with ZN concentrates preparation. However both preparations showed 5.6% of positive cases with a significant relationship between both techniques (P= 0.0000). This study also found that 20% of cases showed false positive with Ziehl Neelsen concentrate technique and 30% showed false positive with

Auramine-O concentrate preparation. In conclusion, there was a highly significant relationship in the performance of ZN and Auramine O techniques in the detection of AFB, although auramine-O showed a greater false positive than ZN method in the detection of AFB. Both ZN and Auramine-O techniques can be used in the detection of AFB in this study population. However, auramine-O should remain a method of choice in this study population whenever dealing with few samples because it showed a greater sensitivity than ZN method in the detection of AFB[21].

Amir Hossein Jafarian et. al., designed a study to compare the sensitivity and specificity of Acid fast and Auramine-Rhodamine staining and Multiplex PCR for the detection of Mycobacterium tuberculosis complex and non tuberculosis Mycobacteria on formalin fixed paraffin embedded tissues (FFPE). Forty cases of FFPE pleural and bronchial tissue with chronic granulomatous inflammation and caseous necrosis and 10 cases with bronchogenic carcinoma as controls were investigated. They designed a Multiplex PCR DNA amplification method with two targets: 123bp DNA fragment from IS6110, which is present only in mycobacterium tuberculosis complex and 162bp DNA encoding Ag 85complex which is present in all of mycobacteria. The FFPE also stained by Acid fast and Rhodamine-Auramine staining method. In 26 samples (65%) 123 bp and 162 bp DNA fragments were detected together (12 in bronchial samples and 14 in pleural samples). The 162 bp fragment wasn't detected alone. The sensitivity of PCR was 65%

and the specificity was 100%. Eleven cases were positive for Acid fast staining. There was 27.5% sensitivity and 100% specificity. Thirteen cases were positive for Auramine-Rhodamine staining; there was 32.5% sensitivity and 100% specificity. All of the 10 controls were negative for 123 bp, 162 bp DNA fragments, for Acid fast and Auramine-Rhodamine staining. This study shows that the sensitivity of auramine fluorescent staining is more sensitive than acid fast staining[22].

Hemalatha Krishnaswami and C.K.Job et. al., designed a study to demonstrate the advantage of fluorescent staining in the diagnosis of tuberculosis in tissue sections.265b consecutive lymph node biopsy specimens were studied over a ten month period. One half of the lymph node was kept in a sterile container for culture studies and the other half was fixed in 10 percent formalin. Several 5 µ sections were made, embedded in paraffin, stained using haematoxylin and eosin stain and studied. All the sections which were histologically diagnostic of tuberculosis were stained for Mycobacterium tuberculosis by the Ziehl-Neelsen stain and Fluorescent stain using auramine and rhodamine. Ziehl-Neelsen stain was done on tissue sections according to the method described in the Armed Forces Institute of Pathology Manual of Histologic and Special Staining Techniques (1937) and acid fast bacilli were looked for by searching the whole section under oil immersion lens using a 10x objective. The fluorescent staining was done by auramine-rhodamine stains. These sections were examined on the day of

staining using a 10x ocular and a low power (10x) objective lens. Characteristic features typical of Mycobacterium tuberculosis were confirmed with a higher power (45x) objective lens. Dark field illumination was preferred as it was less fatiguing and also in its fluorescence, the contrast between organisms and the background was more prominent. Control sections that were known to contain organisms, were prepared with each group of unknowns. An additional negative control of 10 different lymph node sections without granulomatous lesions were stained with the fluorescent dye and were found to be negative. Histopathologically 128 lymph nodes were diagnostic of tuberculosis out of 265 lymphnodes. Mycobacterium tuberculosis was found in 91 specimens (71.1 per cent) using Ziehl-Neelsen stain and in 102 specimens (79.7 per cent) using fluorescent stain. With fluorescent stain the bacilli fluoresced a reddish golden yellow while the tissue appeared a dark pale green and the background appeared black. Artefacts tended to appear hazy yellow or grey green and lacked the reddish tinge and were poorly delineated. Although the organisms tended to appear larger than expected due to fluorescent glow, they retained their slightly curved rod like structure. Out of 265 lymph nodes subjected to culture for acid fast bacilli, mycobacterium tuberculosis was grown in 101 specimens. Of the 128 histopathologically positive biopsies, 101 were identified by culture, 102 by fluorescent method and 91 by Ziehl-Neelsen staining method. Hence it was stated that where culture facilities are not available, the tissue sections can be studied using the

simple acid fast staining method according to Ziehl-Neelsen, yielding fairly comparable results. The fluorescent stain has a definite advantage in speed in identification of small numbers of mycobacteria in tissues[23].

Kumar N et al, compared Ziehl-Neelsen and fluorescent staining methods in cytodiagnosis of tuberculosis without classical features. Fine needle aspirates (FNA) were obtained from lymph node and other sites in 250 suspected cases of tuberculosis. Twenty-four cases proved to be non-tubercular on FNA smears and served as negative controls. Of the smears obtained from the remaining 226 cases, 233 were classified into five groups based on cytomorphological features, i.e. presence of necrosis and granulomas, necrosis alone or acute inflammatory exudate (AIE) with or without granuloma. Cases with AIE alone formed the largest group (n = 123). Staining for AFB was done by Ziehl-Neelsen (ZN) and fluorescent methods in all 250 cases. A correlation of AFB positivity and its semiquantitative scoring (1+ to 3+) with the cytomorphological spectrum was done. Overall AFB positivity by ZN staining was 33.5% and by fluorescent staining 45.4%. When the two methods were combined, AFB positivity was 58.7%. Fluorescent staining was superior to the ZN stain in the presence of a low bacterial load as seen in smears with diagnostic cytomorphological features of tuberculosis. In problem areas like AIE alone or with occasional granulomas, AFB positivity by ZN staining is nearly as good as the fluorescent method, because the bacterial load is high[24].

Masood Ziaee et al, compared the diagnostic value of fluorochrome microscopy (FM) with Ziehl-Neelsen (ZN) staining in the diagnosis of tuberculosis. In this study, 920 consecutive patients suspected of having pulmonary TB were selected. A total of 2760 sputum specimens were collected from them between April 1996 and April 2004. All samples were smeared and stained using both Ziehl-Neelsen and auramin-phenol methods as recommended by WHO. Two independent experts examined smears microscopically. The American Thoracic Society recommendation was used for reporting the results of smears examination. All positive smears by fluorescent microscopy were over-stained by ZN technique for confirmation. Positive and negative control slides were included with each staining batch for internal quality control of the staining methods. Active TB was diagnosed in a patient when two sputum specimens were positive for AFB by smear. Smear negative patients with history of prolonged fever, weight loss and/or cough, and radiological evidence suggestive of TB, were also considered to have clinically diagnosed TB. The results obtained from ZN smears and the auramine-phenol methods were compared together. Sensitivity, specificity, and positive and negative predictive values for each method, with their 95% confidence intervals, were calculated using SPSS 10 software. A total of 102 out of 920 study subjects had pulmonary TB, and among them 68 (66.66%) patients were smear positive by either staining method while others were smear negative. The smear results obtained from both staining methods are

compared. The agreement in grading between the two methods was 93.2%. The proportion of positive smears detected was 51%and 57% for the ZN and auramine phenol staining methods, respectively. ZN method missed 16 (27.6%) of the 58 slides found positive by the auramine phenol method while auramine phenol method missed only 10 (19.2%) of the 52 slides found positive by the ZN method. The performance of the ZN method and auramine phenol method were evaluated using a combination of smear result and clinical picture of patients as the “gold standard.” The proportion of smear negative patients was higher with the ZN (49.02%) than with auramine phenol method (43.14%). The ZN method missed 6 more patients than the auramine phenol method did. The sensitivity, specificity, positive predictive value and negative predictive value were 51%,100%,100%,94% and 57%,100%,100%,95% for the ZN and auramine phenol staining methods, respectively. They also compared the sensitivity of ZN staining in different contamination conditions. The results showed that in 1+, 2+ and 3+ contamination, the sensitivity of ZN staining was 70%, 67% and 83%, respectively. In conclusion, it was said that, because of the higher sensitivity and rapidity of the fluorochrome technique compared to ZN, in clinical laboratories with large specimen numbers, at first it is preferred to evaluate smears with fluorochrome staining and then positive specimens should be confirmed by ZN staining[25].

Laifangbam S et al, did a comparative study of fluorescent microscopy with Ziehl-Neelsen staining and culture for the diagnosis of pulmonary tuberculosis. This comparative study was conducted on the sputum specimens of 102 patients suspected of pulmonary tuberculosis. Patients attending the Respiratory Diseases Department OPD and the DOTS Centre, RIMS, and having fever, night sweats, cough for more than 3 weeks with sputum, loss of appetite, loss of weight, chest pain, haemoptysis and/ or radiological evidence of tuberculosis were included.

Those cases who have not taken a course of antibiotics, known cases of carcinoma lung and paediatric cases were excluded. Those unable to produce at least 5 ml of mucopurulent sputum were also excluded. 3 sputum samples were collected on 2 consecutive days from each patient - spot specimen on the first day, one early morning and one spot specimen on the second day. Samples were collected in clean, sterile, leak-proof, wide-mouth containers. The processings of the samples were carried out in a biosafety cabinet. Each sample was processed by the Petroff's method and subjected to Ziehl-Neelsen (ZN) staining, Fluorescent Auramine-O (AO) staining and culture on modified Lowenstein-Jensen medium. Smear reporting is done according to Forbes BA et al. Out of 102 clinically diagnosed pulmonary tuberculosis patients, 45, 73 and 72 cases were found to be positive for AFB by ZN staining, AO staining and culture techniques respectively. The ZN smear positivity rate and the AO smear positivity rate in this study was 44.1%

(45/102) and 71.6% (73/102) respectively. The combined smear positivity using both the staining techniques was 72.5% (74/102). Scores were definitely higher by fluorescence microscopy: 73 (20+28+16+9) positive as against 45 (3+19+16+7) positive by the ZN method. The difference in the case-yields was found to be highly significant ($p < 0.001$). Disregarding the scores, 72 (28+44) of 102 smears gave identical results. In other words, there was 70.6% agreement or 29.4% disagreement between ZN and AO. In ZN stained smears, 42 (19+16+7) multibacillary and 3 paucibacillary cases were detected whereas in fluorochrome stained smears 53 (28+16+9) multibacillary and 20 paucibacillary cases were detected. 43 out of 72 culture positive cases were diagnosed by ZN stained smear microscopy. 29 cases missed by ZN were detected by culture. There was agreement in 71/102 cases (69.6%) and disagreement in 31/102 (30.4%). There was agreement in 97/102 cases (95.1%) and disagreement in 5/102 (4.9%) between fluorescence microscopy and culture. On comparison against culture, the gold standard in the detection of the tubercle bacilli, the sensitivity and the predictive value of negative test of the ZN stain was much lower than those of AO stain. The false positive results of the AO stain were slightly higher than that of ZN. The false negative results of the ZN stain were much higher than that of AO. Combining the results of ZN and AO, the efficiency was significantly increased than that of the individual stains. It was concluded that fluorochrome stain is more efficient over ZN stain in detecting TB bacilli in

sputum, especially the paucibacillary cases. Since screening is done under lower power of magnification (400x), fluorescence microscopy has been found to be less time consuming as compared to ZN method (1000x) in the diagnosis of tuberculosis. Hence, it has been advocated to be a method of choice where a large number of sputum smears are to be examined. The fluorescing bacilli are easily identifiable and cause less eye strain. Culture examination is more reliable but is time consuming, expensive and requires trained technical hands. The efficacy of fluorescence microscopy proved to be much higher than conventional light microscopy and is comparable to that of culture[26].

Niaz Mohammad Sulaiman Khail et al., did a morphological study of tuberculous lymphadenopathy using both Ziehl-Neelsen and fluorescent stains. Two hundred patients with lymphadenopathy were screened and one hundred and one patients with tuberculous lymphadenopathy were studied. Their ages ranged from 2-70 years. Maximum numbers of cases were in age groups 10-29 years. Females(69.31%) were more affected than males(30.69%). The common presenting symptom was fever. Out of 101 patients 83 had affected cervical lymph nodes, 7 had axillary lymph nodes and 11 had multiple sites of lymph node involvement. Fluorescent staining of histopathological sections from 103 chronic granulomatous lymphadenitis gave positive results in 76 out of 103(73.78%) cases, however Ziehl-Neelsen staining was positive only in 29 out of 103(28.15%) cases. The yield of

mycobacteria on fluorescent staining was highly significant ($p<0.001$) as compared to Ziehl-Neelsen staining thereby providing the superiority of fluorescent stain[27].

Khagi AR et al, did a cross sectional study to demonstrate comparison of different diagnostic method for mycobacterium tuberculosis in suspected patients. A total of 250 samples were included in the study. Ethical approval and consent of patient was taken. Sputum is the sample of choice in this study. The samples were divided into group A by sputum smear positive by Auramine fluorochrome stain(n=150), one from each patient and group B by sputum smear negative by Auramine fluorochrome stain (n=100), one from each patient. The sputum sample was subjected to direct microscopy examination by Ziehl-Neelsen and Auramine fluorochrome method. In ZN staining the Acid fast bacteria (AFB) appear red and they were reported and recorded according to the Bulletin of the International Union Against Tuberculosis (IUATLD) 1978 WHO. Similarly in auramine method the reporting and recording was done according to the ALA scale (American Lung Association, USA). Another part of the sample was used for primary culture by Lowstein-Jension method. The inoculated slants were placed in the incubator at 37°C. The caps were closed tightly when the surface of the media dried; incubation was continued up to at least 8 weeks. The culture was observed at the 7 day for the rapid growers and at fourth week for slow growers.

Presence of colonies on the medium was checked when colonies were present at each stage (at 7th day or 4th week); their acid fastness was determined by ZN staining. If the colonies do not appear at the time mentioned above, observe weekly until 8 weeks before giving decision as negative. Recording and reporting by was done according to WHO guidelines. Chest X-ray was taken by radiographer technologist of NTC and reading of X-ray was done by expert of NTC. In National Tuberculosis Centre, mainly mass miniature Xray film was taken for tuberculosis of lung, which could be used to diagnose PTB with certain characteristics of chest radiograph. In the study group A (n=150) all the specimens were positive in Auramine fluorochrome stain and all of them show positive in X-ray but only 134 showed positive in Ziehl-Neelsen stain and 136 showed positive in culture. In the study group B (n=100), all the specimens were negative in Auramine fluorochrome stain and all of them show negative in Ziehl-Neelsen stain but 14 of them were positive in culture and 24 were positive in chest X-ray. The study was not verified by PCR. The group A includes 150 direct Auramine fluorochrome smear positive sputum samples, 90.66% of them were positive in cultural examination in LJ medium, 89.33% were positive in Ziehl-Neelsen stain and all the specimens i.e.100% were positive in radiological examination.

Among the studied 150 Auramine fluorochrome stain positive cases, 82% (n=123) were male and 18% (n=27) were female. This study showed that

the highest number was seen in the age group 41 to 50 (26.66%), followed by 31 to 40 (24%).

Out of 150 Auramine fluorochrome positive cases 123 (82%), were male and 27 (18%) were female. This greater occurrence in male than female is statistically significant(x2=61.44). Among the studied 150 Auramine fluorochrome stain positive cases, only 136 cases were positive in culture, in which 82% (n=111) were male and 18% (n=25) were female. This study showed that the highest number was seen in the age group 31 to 40 (26.47%), and 41 to 50 (26.47%), followed by 21 to 30 (18.38%) . Among the studied 150 Auramine fluorochrome stain positive cases, all 150 cases were positive in X-Ray, in which 82% (n=123) were male and 18% (n=27) were female. This study showed that the highest number was seen in the age group 41 to 50 (26.66%). Out of 150 Auramine Fluorochrome positive cases 123 (80%) were male and 27 (18%) were female. This greater occurrence in male than female is statistically significant (x2=61.44). Among the studied 150 Auramine fluorochrome stain positive cases, all 134 cases were positive in Ziehl-Neelsen stain, in which 82% (n=123) were male and 18% (n=27) were female. This study showed that the highest number was seen in the age group 41 to 50 (27.61%), followed by 31 to 40 (26.86%), 21 to 30 (15.67%), 11 to 20 (14.92%) and so on. Out of 134 Ziehl-Neelsen stain positive 110 (82%) were male and 24 (18%) were female. This greater occurrence in male than female is statistically significant (x2= 55.18). In group B, out of 100,

Auramine fluorochrome smears negative sputum samples, 24% (24/100) were found to be positive by X-ray and the remaining 76 % (76/100) were negative, where all fluorochrome stain positive samples showed positive in ZN stain.

Among 100(100%) Auramine fluorochrome staining negative sputum samples collected from these cases, 14% (14/100) samples were positive by culture in LJ medium where as the remaining 86% were negative. Since all the Auramine fluorochrome stain positive cases showed positive in X-ray and all the Auramine fluorochrome stain negative cases showed negative in ZN stain, the Auramine fluorochrome stain positive cases do not require to do X-ray examination and Auramine negative cases do not require to do ZN stain, it saves time and money but some of the Auramine negative cases showed positive in culture. Hence the study concluded that the diagnosis of PTB could make by Auramine fluorochrome microscopy and culture. Not all X-ray shadows, collapse, cavities etc should be treated for PTB unless sputum is positive. Depending upon other supporting symptoms further investigations are recommended[28].

Aggarwal P et al., did a clinico-bacteriological study of peripheral tuberculous lymphadenitis. A total of 138 patients with tuberculous lymphadenitis were included in the study. Diagnosis of tuberculosis was established on the basis of fine needle aspiration cytology, histopathology, presence of mycobacteria on Ziehl Neelson stain or auramine rhodamine

stain, or aspiration of pus with negative Gram's stain and pyogenic culture with radiologic evidence of pulmonary tuberculosis. Mycobacterial cultures were performed on aspirated material and species identified using standard methods. Of 138 patients, single lymph nodal enlargement was found in 48.6% patients while others had more than one lymph nodes. Lymph nodes were matted in 26.8% cases while fluctuation could be elicited in 12.3% patients. Chest X-ray showed evidence of active pulmonary lesions or mediastinal lymphadenopathy in 28.3% cases. The fine needle aspiration cytology was positive for tuberculous lymphadenitis in 41.3% cases while it revealed granulomas or necrosis in another 13% cases. The Ziehl-Neelson and the auramine-rhodamine staining were positive in 19.6% and 26.8% patients, respectively. On culture, the lymph node aspirate was positive for Mycobacterium species in 40.6% patients. In all but two cases, the culture revealed presence of Mycobacterium tuberculosis. The other two cultures revealed growth of Mycobacterium fortuitum chelonae complex. Of the two HIV-positive patients, Mycobacterium tuberculosis could be isolated in one case. This study also showed that sensitivity of auramine staining is more than that of ziehl-neelsen staining[29].

R Pahwa et al., did an assessment of possible tuberculous lymphadenopathy by PCR compared to non-molecular method like various staining and culture. The objective of this study was to compare the various diagnostic techniques in clinically suspected cases of tubercular lymph nodes

and to find a suitable, cost-effective but sensitive and specific method for diagnosis. A total of 100 cases were recruited for the study. Fine needle aspiration cytology was done in all cases and the smears prepared were processed for Giemsa, Ziehl-Neelsen's, Kinyoun and Papanicolaou stains. Parts of the aspirated materials were assessed by fluorescent staining, culture and PCR. Seventy-four percent of aspirates were positive by fluorescent stain while only 22 % were positive by culture. PCR could be performed in 55 cases, out of which 22 (40 %) were positive. When compared to culture, the sensitivity and specificity of PCR were found to be 89.5 % and 86.1 %, respectively. Fluorescent stain was found to be the most sensitive (81.8 %) of the conventional methods but showed poor specificity (28.2 %). Interestingly, PCR detected 80 % of smear-negative but culture-positive cases[30].

Kumar VA and Chandra PS et. al., did a study on auramine phenol staining of smears for screening acid fast bacilli in clinical specimens. The aim of this study was to find out the value of auramine phenol (AP) staining technique in diagnosis of the suspected tuberculosis cases. A total of 2000 samples which included sputum (746), gastric aspirates (380), urine (336), endometrial biopsy (150), pleural fulids (146), synovial fluids (67), ascitic fluids (35), cerebrospinal fluids (43), bone marrow (18), lymph node biopsy (11), pericardial aspirates (6), skin biopsy (4), peritoneal fluids (2), and stool (1) were included in the study. Sample were subjected for decontamination procedure by using standard Petroffs method. The deposit smears were

stained by auramine phenol (AP stain) and Ziehl-Neelsen staining (ZN stain) and specimens were cultured for Mycobacterium tuberculosis. Of the total positive isolates 69.23% were having pulmonary tuberculosis and 30.76 had extrapulmonary tuberculosis. Genitourinary tuberculosis was the most common diagnosis among the extrapulmonary tuberculosis followed by chronic synovitis, bursitis, meningitis, septic arthritis and pericardial effusion. Out of 130 positive samples 70 by culture, 66 smears were positive by auramine phenol stain and 62 were positive by ZN stain. A total of 27 samples were tested positive only by AP staining technique, which included (12) pulmonary and (15) extrpulmonary samples. The endometrial biopsy and pericardial fluid samples showed positive for acid fast bacilli by AP stain only, whereas ZN stain and culture technique failed to demonstrate any bacilli in the same sample. Auramine stain showed high sensitivity (47.14%) and specificity (96.58%). Result of the present study showed that the auramine stain is a better method for screening samples from the suspected cases of tuberculosis sample especially pulmonary and extrapulmonary cases where bacilli count is usually low[31].

Hussain Gad ElKarim Ahmed et al., did a retrospective descriptive study on to investigate the morphological pattern of tuberculous lymphadenitis, as well as to assess the reliability measures of (ZN) Ziehl-Neelsen and fluorescent methods in identification of *Mycobacterium tuberculosis.* One hundred lymph node biopsies were retrieved from 631

lymph node biopsies, which were previously obtained from patients with enlarged lymph nodes. All specimens were formalin-fixed paraffin wax processed tissues. Information regarding each patient was obtained from each patient's file. The specimens were fixed in 10% formalin and then processed by tissue processing machine using the following schedule adopting 24-hour scheduling. Three 5-microns thickness sections were obtained from each patient's block using Rotary Microtome. Of the 3 sections, each one was stained with one staining procedure (haematoxylin and eosin, ZN, or fluorescence). In this descriptive study, we assessed the histopathological pattern of TB in 100 tuberculosis patients, their ages ranging from 7 to 86 years with a mean age of 29 years old. The great majority of the specimens were obtained from cervical lymph node followed by axillary lymph node representing 74 (74%) and 9 (9%), respectively. The remaining sites include mesenteric, inguinal, mediastinal, and submandibular, constituting 6 (6%), 5 (5%), 4 (4%), and 2 (2%) correspondingly. In this study, 100 patients with enlarged lymph nodes were diagnosed as having lymph node tuberculosis by histopathology. These patients were further divided into two groups according to the presences of strong and weak tuberculosis histopathological evidences. Those showing histopathological pattern containing giant cells + granuloma + caseation were considered as strong evidence, and the other showing less evidences (e.g., ill-defined aggregates of epithelioid histiocytes only, palisading granulomas without necrosis and giants cells, etc.) were considered

as weaker evidence. Accordingly, the strong evidence (positive) was used as a gold standard for comparing the other variables. Accordingly, of the 100 patients, 68 were categorized as having strong evidences (positive) and the remaining 32 were detected with weaker evidences (positive), cases. Of the 100 studied lymph nodes, only 3 (3%) were demonstrated as positive in ZN (in all cases more than 5 bacilli were seen). The entire 3 ZN positive were previously found as strong evidence positive. On staining of the lymph node by fluorescent method, 9 (9%) were found positive for *M. tuberculosis*. Out of the 9 positive, 7 (7%) were identified as strong positive and the remaining two were at negative level. This study also concluded that fluroscent staining is more sensitive than ziehl-neelsen[32].

N P Singh, S C Parija et. al., have compared light microscopy of ZN stained smears with that of fluorescence microscopy of sputum smears stained by auramine-phenol flurochrome dye for detection of AFB in sputum specimens. Sputum specimens from a total of 2,600 clinically suspected and diagnosed cases of pulmonary tuberculosis were examined by both the methods. Sputum specimens from a total of 1,104 patients were found to be positive for AFB. These included sputa from 975 (37.5%) patients positive for AFB by both ZN and auramine staining methods and sputa from an additional 129 (4.96%) patients positive for AFB by auramine staining only. Thus, it was concluded that auramine staining of sputum smears in comparison to that of ZN staining is a better method of sputum microscopy for demonstration of

AFB in sputum specimens. Fluorescence microscopy is relatively more sensitive and has the added advantage of allowing a large number of sputum specimens to be examined in a given time, in laboratories equipped with a fluorescent microscope[33].

Alan G Cheng et al., evaluated the effectiveness of the auramine orange (AO) stain in diagnosing mycobacterial cervical adenitis (MCA) from fine needle aspiration (FNA) cytology. A retrospective review of 19 patients evaluated at 2 urban hospitals from 2000 to 2003 for suspected MCA. FNA specimens were inoculated to culture media and had direct smears stained by the auramine acid fast method. Mycobacteria were identified in 16 (84.2%) of 19 AO-stained FNA specimens, with results available within 4 hours. Corresponding cultures were positive for mycobacteria in 12 specimens, 9 tuberculous and 3 nontuberculous, and grew Mycobacterium tuberculosis from the 3 AO-negative specimens. Three of the 4 patients with negative cultures had previously taken anti-mycobacterial medications. This study also concluded that the AO stain with fluorescence microscopy is a sensitive and rapid method for detecting tuberculous and nontuberculous mycobacteria. It is a valuable tool for the otolaryngologists and pathologists in the diagnosis of MCA[34].

Gülnur Tarhan et al., compared auramin-rhodamine (A-R) and Erlich-Ziehl-Neelsen (EZN) staining techniques for the diagnosis of

tuberculosis. Of 311 sputum samples collected from active pulmonary tuberculosis patients and tuberculosis-suspected patients, 103 (33%) were found culture positive. In the direct microscopic examination of EZN stained smears, 86.4%(89/103) of culture positive samples, and 3.8%(8/208) of culture negative samples yielded asido-resistant bacteria, while these rates were 74.8%(77/103) and 11.5%(24/208) for A-R staining method. When culture was accepted as reference method, the specificity and sensitivity of the staining techniques were found as 88.5% and 74.8% for A-R, and 96.2% and 86.4% for EZN, respectively. As a result, it was concluded that, the use of A-R staining alone, could not be an alternative method to EZN staining[35].

Trusov, A., R. Bumgarner, et al., did cross-sectional studies in Russia (n = 502) and Macedonia (n = 205), with fluorochrome-stained sputum examined by 1) the new Lumin light emitting diode (LED) fluorescent attachment on a light microscope, and 2) conventional fluorescent microscope (CFM) available in each laboratory, and compared to 3) Ziehl-Neelsen (ZN) restaining/reading of the same smears. They did comparison of all the above three methods. In Macedonia, the sensitivity of the Lumin and CFM were 87.8%, and that of restained ZN smears with conventional light microscope was 78.0%. In Russia, sensitivity was as follows: Lumin 72.8%, CFM 52.5%; re-stained ZN smears 28.5% and directly ZN stained smears 55.6%. It was concluded that fluorescence microscopy is more sensitive than conventional microscopy. The Lumin attachment to conventional light microscopes

provided results equal to or better than the CFMs. Smear restaining for ZN showed a 12% advantage for Lumin and CFM in Macedonia, in line with other meta-analyses. Restaining for ZN gave poor results in Russia for unknown reasons. Retrospective analysis of directly ZN-stained smears showed 55.6% sensitivity compared to the Lumin (72.8%) [36].

Kommareddi, S., C. R. Abramowsky et al., did a study to compare the fluorescent auramine-O and Ziehl-Neelsen techniques in tissue diagnosis of Nontuberculous mycobacterial infections. Biopsy specimens from 22 patients with clinical histories highly consistent with nontuberculous mycobacteriosis in which part of the tissue was cultured were selected for study. Coded tissue blocks and control specimens were stained by the Ziehl-Neelsen (ZN) or auramine-O (A0) fluorescent technique and examined blindly for the presence of characteristic organisms. Results of these studies were compared with the culture results, and predictive values were calculated. This experience showed that the AO technique is technically simpler, allowing faster screening at lower power and showing greater sensitivity and predictive value of a negative result although less specificity than the ZN technique[37].

Hooja, S., N. Pal et al., compared Ziehl Neelsen & Auramine O staining methods on direct and concentrated smears in clinical specimens. Smears were prepared from 393 clinical samples both by direct and after

Petroff's concentration and examined by fluorescent microscopy and Ziehl Neelsen method. The concentrated material was also cultured on Lowenstein Jensen media and the results of the two microscopy methods were compared with the culture results taken as the gold standard. Mycobacterial growth was detected in 137 (35.77%) specimens, out of which three were non-tubercular mycobacteria. Using culture as the reference method, the sensitivity of direct staining was 55.55% for ZN and 71.85% for AO. Direct fluorescent microscopy detected 9.29% paucibacillary sputum samples that were missed on ZN staining. On concentration, the sensitivity increased by 6.67% for ZN and 11.11% for AO. The sensitivity of AFB smear microscopy increased by 27.41% and was statistically significant (p = < .001) when both methods were combined. The specificity was 99.19% for both ZN and AO. Thus, fluorescent microscopy has higher sensitivity and comparable specificity which is further enhanced by concentration[38].

N. Greenwood and H. Fox et. al., compared Ziehl-Neelsen stain with three alternative methods of staining tubercle bacilli in paraffin sections: Fite's method; a modification by Armstrong and Price of Fite's method; and a fluorescent method using the auramine-phenol stain. Seventy cases which had been diagnosed as tuberculosis were selected at random from the files of the Department of Pathology, University of Manchester. Sections from all these cases showed epithelioid cell granulomata and areas of caseation. The tissues were from a variety of anatomical sites including lymph nodes (21), kidney

(9), gastrointestinal tract (6), epididymus (5), liver (4), omentum (4), synovium (3), brain (1), vagina (1), heart (1), spleen (1), and skin (1). Sections from each case were cut and stained by all four methods. All sections were examined initially using a 40x objective and 10x eyepieces. They were then reexamined using 100x oil-immersion objective and 10x eyepieces. Sections stained by the fluorescent method were also examined using a 25x objective and 10x eyepieces. The yield of positive results was much greater with the fluorescent method than with the other techniques. The sections in which a positive result was obtained solely with the fluorescent method showed only a very few bacilli. The fact that none of the fluorescent negative cases were found to be positive by any of the other methods suggests that it is a reliable technique and is unlikely to give false negative results. The fluorescent method, with its low incidence of false-negative results and its ease of performance, can be used as a screening test. Fluorescent-negative sections can be reported as such, but positive sections can be confirmed by staining the same sections using the Ziehl-Neelsen method and concentrating on the area of the section shown to be positive by the fluorescent method, using, if necessary, the 100x oil immersion objective lens. This study concluded that there is a very strong case for the introduction of the auramine phenol fluorescent technique as a routine tool in diagnostic histopathology[39].

Seth W. Gilkerson And Oscar Kanner et. al., in two sets of experiments, tested the sensitivity and the specificity of the proposed method. In experiment 1, a random group of 400 sputa from tuberculosis wards were processed by both the Kinyoun's acid fast and the phenol-auramine stains. Culture and guinea pig inoculations were made from each specimen. The results of the acid-fast staining were unknown to the observer until the result of the fluorescent stain had been reported. The fluorescent staining yielded 105 positives compared with only 60 by the acid-fast staining. All the 105 fluorescent positives yielded positive cultures, positive guinea pigs, or both. To confirm or refute this apparent specificity, a second experiment was performed with a view to an increased exposure to the danger of false positives. In this series, sputa from tuberculosis and nontuberculosis wards were used. The sequence was randomized according to a table of random digits, leaving the investigators ignorant of the origin of any of the specimens as well as of the results of the acid-fast staining until they had reported their results. The results of the second series of 400 show, with respect to the 207 specimens from the tuberculosis wards, results in keeping with the results of the first series. The fluorescent staining revealed 63 positives against 41 by the acid-fast staining. However, in four cases the fluorescent stain was reported negative whereas Kinyoun's stain showed acid-fast bacilli. The results with the specimens from the nontuberculosis wards lend further support to the stipulation of specificity. Of the 193 cases only 2 were positive,

both by acid-fast and fluorescent staining. This was the first indication of subsequently confirmed tuberculosis. In conclusion, the proposed fluorescent staining method is more sensitive and not less specific than Kinyoun's acid-fast staining.

Thus, from the above studies it is understood that fluorescent stains are more rapid and more sensitive in the detection of acid fast bacilli in various body fluids when compared with routine ziehl neelson method which is commonly employed. The number of fields scanned in the detection of AFB using fluorescent stains is considerably less and the ease of detection of AFB is more when compared with conventional ziehl nelson stain.

In this study, the detection of AFB in suspected cases of tuberculous lymphadenitis by fine needle aspiration cytology is determined using ziehl nelson method and fluorescent method. The sensitivity in the detection of AFB by ziehl neelson and auramine stains in TB lymph node aspirates are compared. The study also helps to provide a definitive and confirmatory cytopathological diagnosis of tuberculous lymphadenitis in H&E proved granulomatous lymphadenitis lesions.

S. NO.	NAME OF STUDY	SPECIMEN	TOTAL NO OF CASES	ZN +VE	AR +VE	RESULTS
1	MYRNA T. MENDOSA ET AL	SPUTUM	2183	132	159	FAM GIVES BETTER OVERALL ACCURACY
2	AMIR HOSSAIN JAFARIAN ET AL	L.N.- BIOPSY	40	11	13	FAM IS MORE SEMSITIVE THAN AURAMINE
3	HEMALATHA KRISHNASAMY AND C.K.JOB	L.N.- BIOPSY	128	91	102	FAM IS FASTER IN IDENTIFICATION OF SMALL NUMBER OF MYCOBACTERIA
4	KUMAR N ET AL	FNAC NODT	250	33.50%	45.40%	FAM IS MORE SENSITIVE AND RAPID THAN ZN
5	MASOOD ZIAEE ET AL	SPUTUM	2760	51%	57%	FAM IS SUPERIOR THAN ZN
6	LAIFANGBAM S ET AL	SPUTUM	102	45	73	EFFICACY OF FAM IS HIGHER THAN ZN
7	NAIZ MOHAMMAD SULAIMAN KHAIL ET AL	L.N.- BIOPSY	103	29	76	YEILD OF MYCOBACTERIA ON FLUORESCENT STAINING IS HIGHER THAN ZN

S. NO.	NAME OF STUDY	SPECIMEN	TOTAL NO OF CASES	ZN +VE	AR +VE	RESULTS
8	KHAGI AR ET AL	SPUTUM	250	134	150	DIAGNOSIS OF MTB IS MADE EASIER BY FAM
9	AGGARWAL P ET AL	FNAC-NODE	138	19.60%	26.80%	SENSITIVITY OF AURAMINE IS MORE THAN ZN
10	KUMAR VA; CHANDRA PS	FNAC-NODE	130	62	66	AURAMINE IS A BETTER METHOD FOR SCREENING MTB
11	HUSSAIN GAD ELKARIM ET AL	L.N.-BIOPSY	100	3	9	FLUORESCENT STAINING IS MORE SENSITIVE THAN ZN
12	NP SINGH ET AL	SPUTUM	2600	975	1104	FAM IS MORE SENSITIVE THAN ZN
13	SETH W. GILKERSON; OSCAR KANNER	SPUTUM	400	105	60	FAM IS MORE SENSITIVE THAN ROUTINE ZN
14	VAMSEEDAR ANNAM	FNAC-NODE	102	45	83	FAM MORE SENSITIVE THAN ZN & CAN BE USED AS ADJUVANT TO H&E

MATERIALS AND METHODS

MATERIALS AND METHODS

Patients suspected clinically of lymphadenopathy with symptoms of TB referred from various departments for FNAC to the Department of Cytology, Stanley Medical College from May 2009 to November 2011 were included in this study. A total of 212 cases of clinically suspected TB lymphadenitis cases were subjected to FNA.

INCLUSION CRITERIA

Inclusion criteria included lymphadenopathy with classical symptoms of TB like evening rise of temperature,chronic cough, unexplained weight loss and PUO.

EXCLUSION CRITERIA

Exclusion criteria included patients without classical features. Patients initially suspected clinically of TB lymphadenitis but later found to be due to reactive lymphadenitis, metastatic secondary deposits and lymphoproliferative disorder by H&E were also excluded.

SUPPORTIVE TESTS

Relevant investigation details like total leucocyte count absolute lymphocyte count, ESR, Mantoux, HIV status, chest radiogram were reviewed in these patients.

PROCEDURE OF FNA

FNAC was performed for all the 212 cases as a OP procedure. Written consent was obtained from the patients and the nature of study was also explained .The patients, after verifying their identification were asked to lie down comfortably and the procedure was explained. After following all the universal safety precautions,examination of the enlarged lymphnodes was done and noted.

FNA was done using sterile disposable syringes. Needle size of 23 for adults and 25 for children were used. A total of 3 smears were taken and smeared on a clean standard glass slide.Cytology running number was marked on the glass slide with a glass marking pencil.Out of the 3 smears taken one was fixed in iso-propyl alcohol for H&E, and the other 2 were heat fixed and air dried by showing the under surface of the slide in a spirit lamp.

All the aspirates by FNAC were processed for direct microscopy using the routine H&E, the conventional ZN staining for the detection of AFB and compared with the findings of the fluorescent method.

THE PRINCIPLE OF Z-N AND FLOURESCENT STAINS.

Acid fast organisms have mycolic acid in their cell walls which are thought to bind fuchsin or fluorochrome stains tightly making them difficult to decolourise with acid alcohol.

Flourochromes are dyes which make the organism fluoresce.The dyes are called fluorophores.

ZIEHL – NEELSEN METHOD:

Reagents- strong carbol fuchsin, acid decolouriser, methylene blue as counter stain.

Procedure

1. Cover the smears with carbol fuchsin.

2. Heat the smear from the lower side of the slide until steam arises for about 8-10 minutes. Do not allow the stain to boil or slide to become dry.

3. Wash with distill water and decolourise for 2 minutes.

4. Wash with distilled water and counter stain with methylene blue for 30 seconds.

5. Wash with distilled water, dry and observe under oil immersion. The smears were screened under oil immersion (1000x) and graded as follows:

0	no AFB seen
doubtful/repeat	1-2 bacilli/300hpf
1+	1-9 bacilli/100hpf
2+	1-9 bacilli/10 hpf
3+	1-9 bacilli/1hpf
4+	10-100 bacilli/1hpf

Results: Acid fast bacilli appear as pink rods in a light blue background.

FLOURESCENT METHOD

PhenolicAuramine O stain commercial preparation with the following reagents was used.

Phenolic Auramine O, conc.Hcl in 70% ethanol, potassium permanganate as counter stain.

PROCEDURE

1. The heat-fixed smears were stained with the phenolic Auramine stain for 15 minutes.

2. The slides were rinsed with distilled water for 2 minutes.

3. Decolorization was performed with 0.5% hydrochloric acid in 70% ethanol for 2 minutes.

4. The slides were rinsed with distilled water for 2 minutes.

5. Counterstaining was performed with 0.5% aqueous potassium permanganate for 2 minutes.

6. The slides were rinsed with distilled water for 2 minutes, and air dried and examined under low power (200x) and confirmed under oil immersion (1000x).

Results:

AFB appeared as green-yellow, slender, rod-shaped bacilli under fluorescence microscope and the smears were graded as follows.

Report	No of AFB observed	
	20x	**40x**
No AFB seen	0	0
Doubtful:repeat	1-2/30F	1-2/70F
1+	1-9/10F	2-18/50F
2+	1-9/F	4-36/10F
3+	10-90/F	4-36/F
4+	>90/F	>36/F

Mycobacterium Tuberculosis culture positive smears were obtained and stained for both ZN & Fluorescent stains and kept as positive controls.

H&E stained lymph node aspirate smears that were reported as reactive lymphadenitis were kept as negative controls.

Controls were stained simultaneously during every batch of staining of test smears.

FLOURESCENCE MICROSCOPE SPECIFICATIONS.

A NIKON make fluorescence microscope with the following specifications was used.

Light source ------- mercury vapour lamp.

Filter set -------- B-2A- BLUE filter.

Excitation -------- 450-490 nm.

Emission -------- > 520 nm.

PRECAUTIONS

The following precautions were strictly followed during the staining procedure.

1. The smears were heat fixed.
2. The smears were covered fully with the stain and was not allowed to get dry.
3. Distilled water was used for rinsing
4. Slides were stained individually taking care that no cross contamination was made.

5. Counter staining was done strictly for two minutes since longer time may quench the fluorescence of AFB.

6. Stains were stored in a dark place and the smears were also examined in a dark room and photographs were taken immediately.

OBSERVATION AND

RESULTS

OBSERVATION AND RESULTS

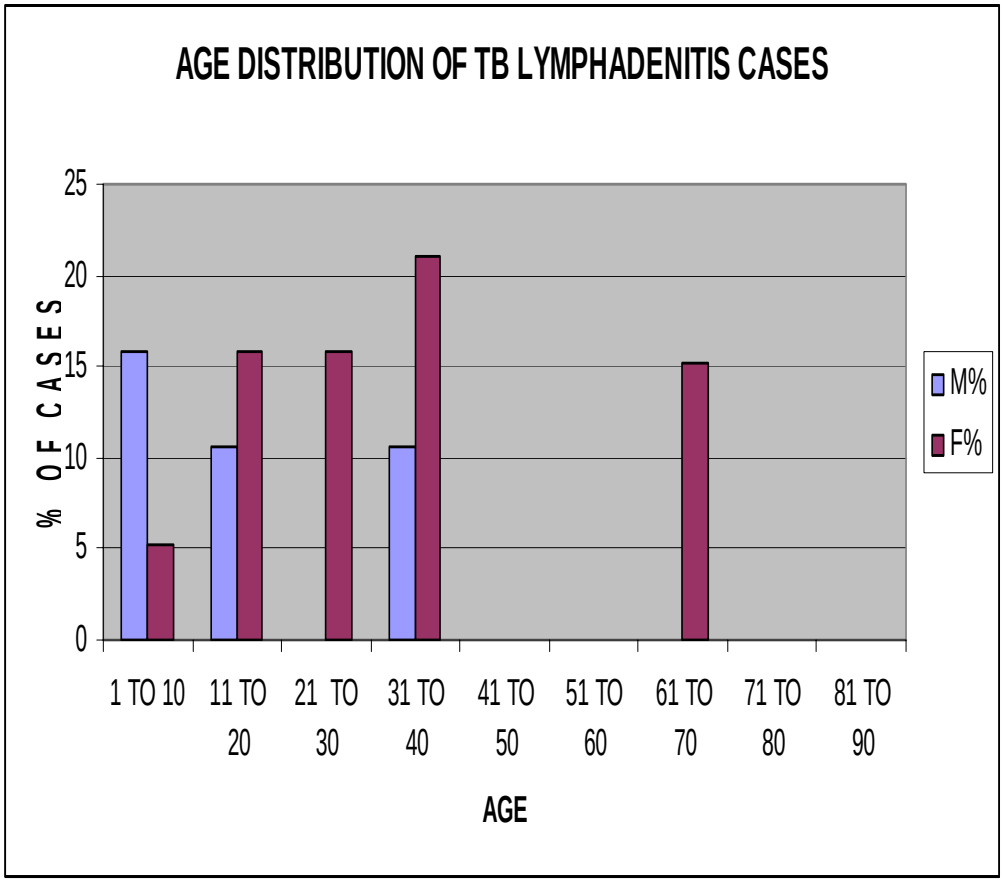
AGE AND SEX DISTRIBUTION OF TB LYMPHADENITIS CASES

AGE	NO OF CONFIRMED AFB+VE CASES		
	M	F	TOTAL
1 TO 10	3	1	4
11 TO 20	2	3	5
21 TO 30	0	3	3
31 TO 40	2	4	6
41 TO 50	0	0	0
51 TO 60	0	0	0
61 TO 70	0	1	1
71 TO 80	0	0	0
81 TO 90	0	0	0
TOTAL	7	12	19

TABLE NO. 1

AGE	% OF CONFIRMED AFB+VE CASES		
	M%	F%	TOTAL%
1 TO 10	15.79	5.26	21.05
11 TO 20	10.53	15.79	26.32
21 TO 30	0	15.79	15.79
31 TO 40	10.53	21.05	31.58
41 TO 50	0	0	0
51 TO 60	0	0	0
61 TO 70	0	15.26	15.26
71 TO 80	0	0	0
81 TO 90	0	0	0
TOTAL%	36.84	63.15	100

TABLE NO. 1a

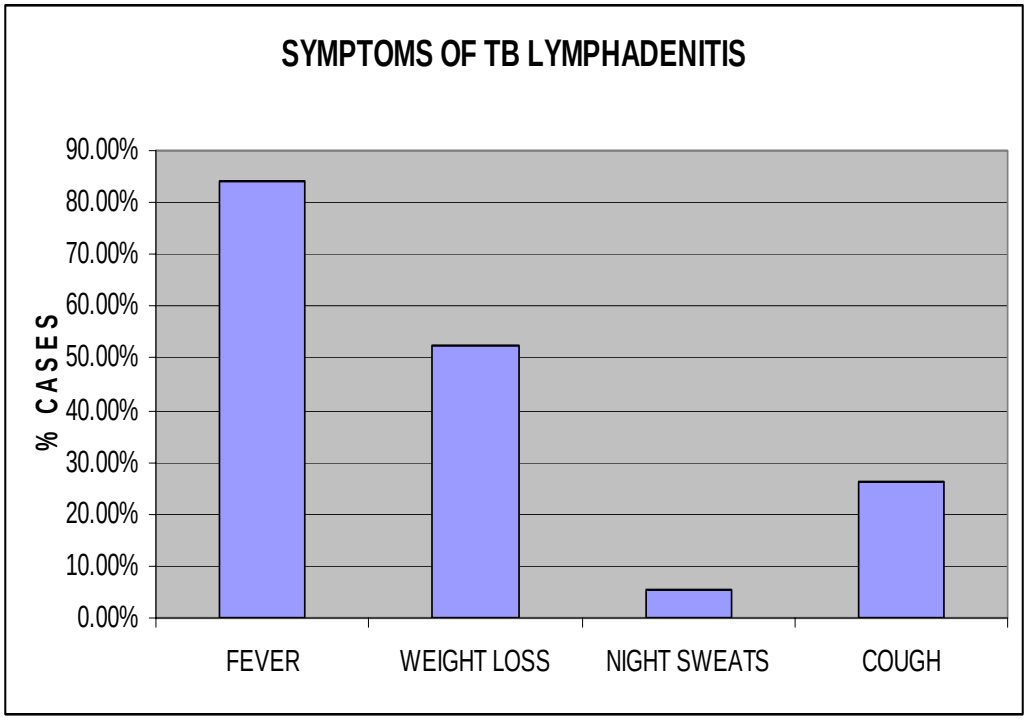


Age range of patients with tuberculous lymphadenitis was from 3 to 70 years. The commonest age group affected was between 31 to 40 years(31.58%). Females were most commonly affected than males(63.15%).

CLINICAL PRESENTATION OF TB LYMPHADENITIS CASES

TB LN- SYMPTOMS	NO OF CASES	%
FEVER	16	84.21%
WEIGHT LOSS	10	52.63%
NIGHT SWEATS	1	5.26%
COUGH	5	26.32%

TABLE NO. 2

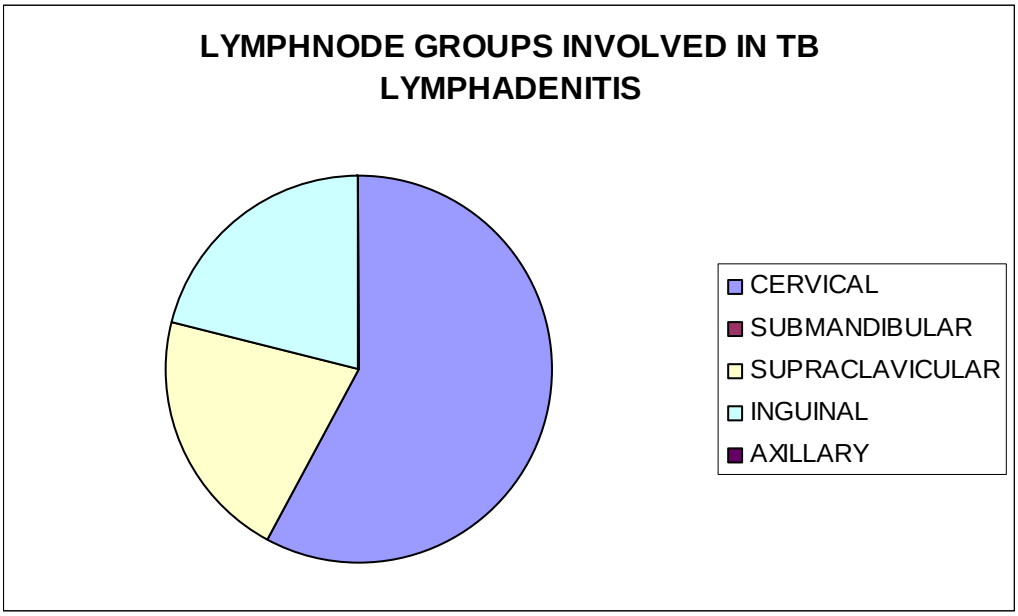


Most common symptom of tuberculous lymphadenitis was found to be fever(84.21%)(evening rise of temperature). Other symptoms were weight loss, cough and night sweats.

LYMPH NODE GROUPS INVOLVED IN TB LYMPHADENITIS

LN GROUP	NO OF CASES	%
CERVICAL	11	57.90%
SUBMANDIBULAR	0	0
SUPRACLAVICULAR	4	21.05%
INGUINAL	4	21.05%
AXILLARY	0	0

TABLE NO. 3



Most common lymphnode group involved in tuberculous lymphadenitis was the cervical group (57.90%). Other lymphnodes found to be involved were supraclavicular(21.05%) and inguinal nodes(21.05%).

CLINICAL CHARACTERISTICS OF A TB LYMPH NODE

TB NODE	NO OF CASES	%
SINGLE	6	31.58%
MATTED	13	68.42%
UL	17	89.47%
BL	2	10.53%
ABCESS	15	78.95%
SINUS	1	5.26%

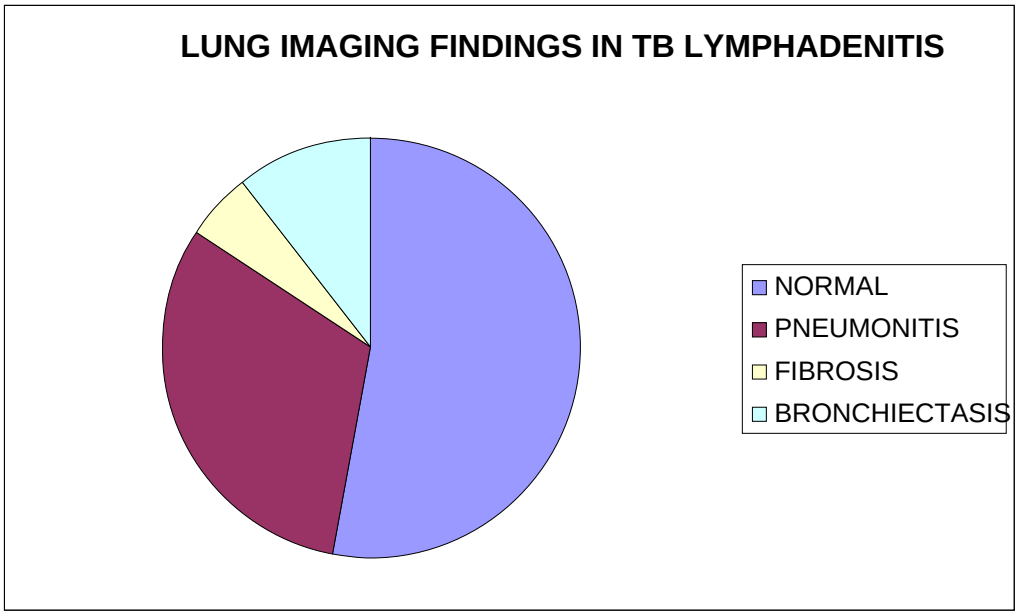
TABLE NO. 4

Most of the cases showed unilateral involvement of nodes(89.47%). Involved lymphnodes were found to be matted in 68.42% of cases. Abcess formation was commonly seen(78.95%).

LUNG IMAGING FINDINGS IN TB LYMPHADENITIS

IMAGING	NO OF CASES	%
NORMAL	10	52.63%
PNEUMONITIS	6	31.57%
FIBROSIS	1	5.26%
BRONCHIECTASIS	2	10.52%

TABLE NO. 5

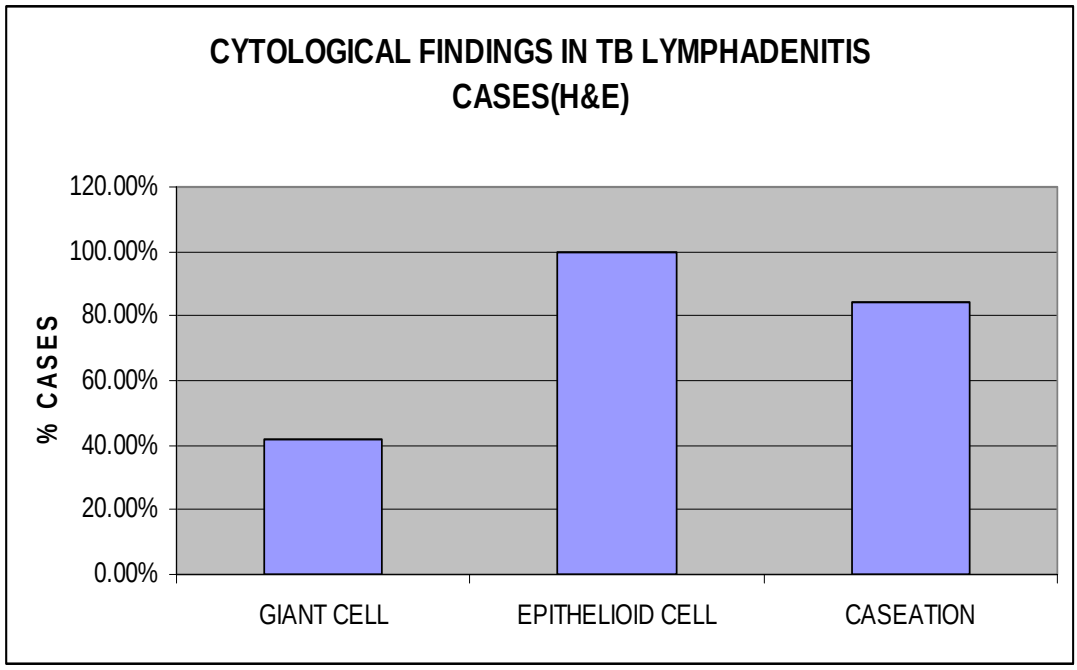


X – ray chest showed no significant findings in most of the cases(52.63%). Findings like pneumonitis(31.57%), fibrosis(5.26%) and bronchiectasis(10.52%) were found in few cases.

CYTOLOGICAL FINDINGS IN TB LYMPHADENITIS (H&E).

H&E	NO OF CASES	%
GIANT CELL	8	42.10%
EPITHELIOID CELL	19	100%
CASEATION	16	84.20%

TABLE NO. 6.

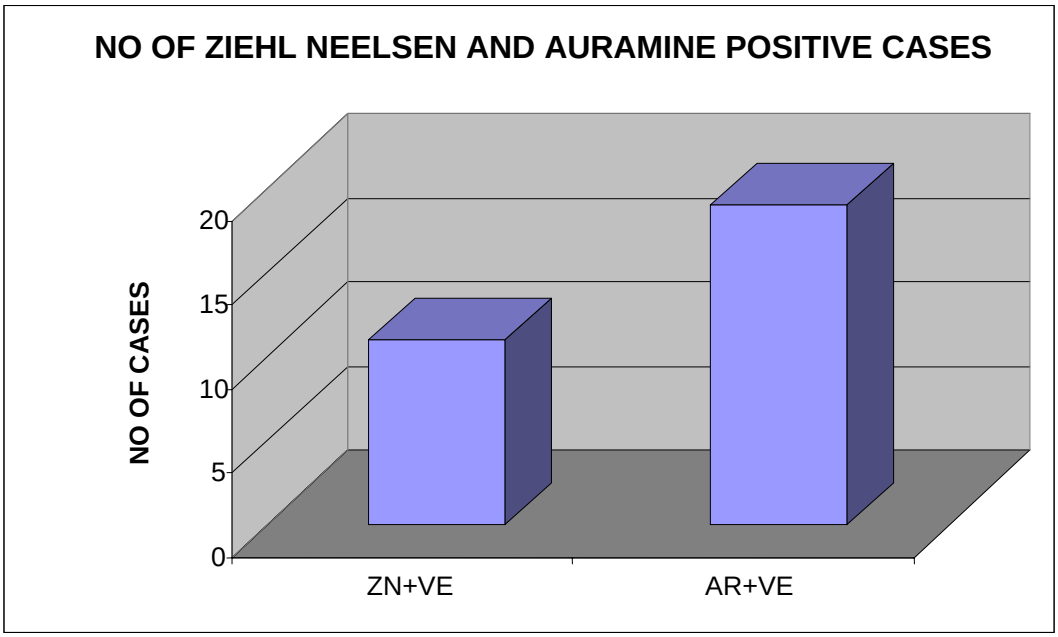


Epithelioid cells were found in fine needle aspiration smears of all the cases of tuberculous lymphadenitis(100%). Giant cells(42.10%) and caseation necrosis(84.20%) were also seen .

NO OF ZIEHL-NEELSEN AND AURAMIINE POSITIVE CASES

STAIN	NO OF CASES	%
ZN+VE	11	11%
AR+VE	19	19%
AR+VE IN ZN-VE CASES	8	8.98%

TABLE NO. 8

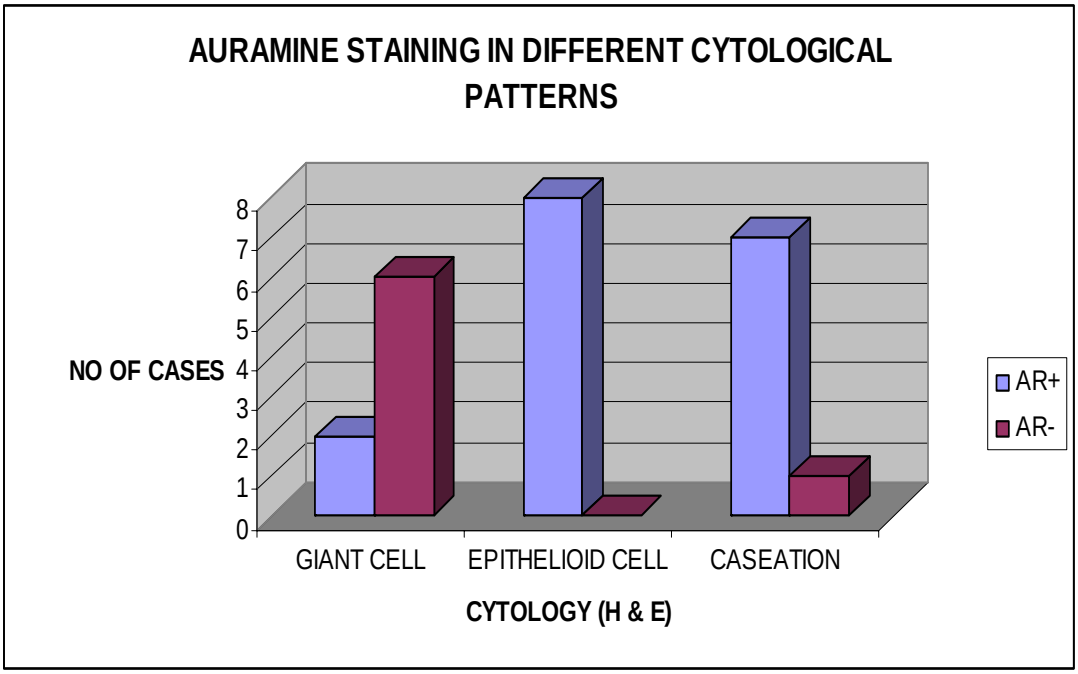


Ziehl Neelsen detected acid fast bacilli in 11 out of 100 cases. Auramine stain showed positivity in 19 cases which included all the 11 ZN positive cases.

AURAMINE STAINING IN DIFFERENT CYTOLOGICAL PATTERNS

CYTOLOGY (H & E)	AR+	AR-
GIANT CELL	2	6
EPITHELIOID CELL	8	0
CASEATION	7	1

TABLE NO. 7



Caseation and epithelioid cells were often associated with positive fluorescent stain.

COMPARISON OF FLUORESCENT STAIN WITH THE GOLD STANDARD ZN STAIN

	ZN POSITIVE	ZN NEGATIVE	TOTAL
AR POSITIVE	11	8	19
AR NEGATIVE	0	81	81
TOTAL	11	89	100

TABLE N0. 9

All the 11 Ziehl Neelsen positive cases were also positive for Auramine stain. Auramine stain also showed positivity in 8 cases which were Zeihl Neelsen negative.

EVALUATION OF ZN AND FLUORESCENT STAIN

	ZN	AO
SENSITIVITY(TRUE+VE)	58%	100%
SPECIFICITY(TRUE-VE)	89%	91%
POSITIVE PREDICTIVE VALUE	100%	58%
NEGATIVE PREDICTIVE VALUE	91%	100%

TABLE NO. 10

The sensitivity of Auramine O stain was more (100%) when compared with ZN stain (58%). The false negativity of ZN stain (42.10%) was considerably more when compared with Auramine stain which means that ZN stain is a less sensitive test when compared with Auramine O(100%).

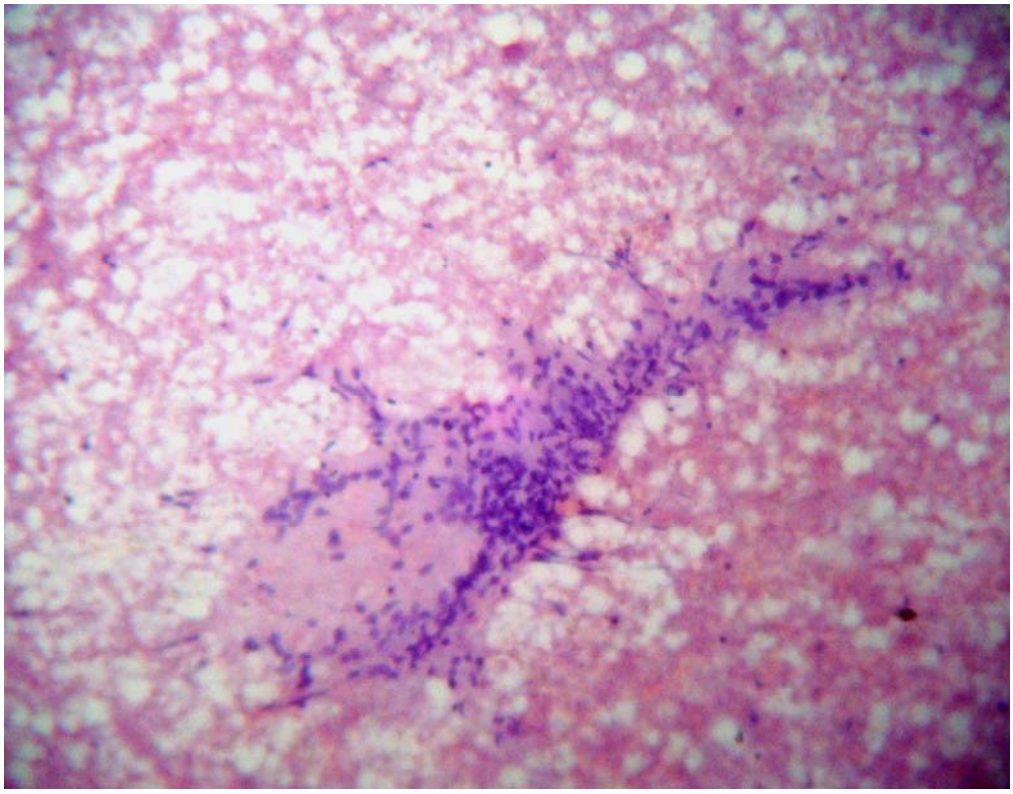


Fig-1- 10X- showing epithelioid cells and lymphocytes. (H&E)

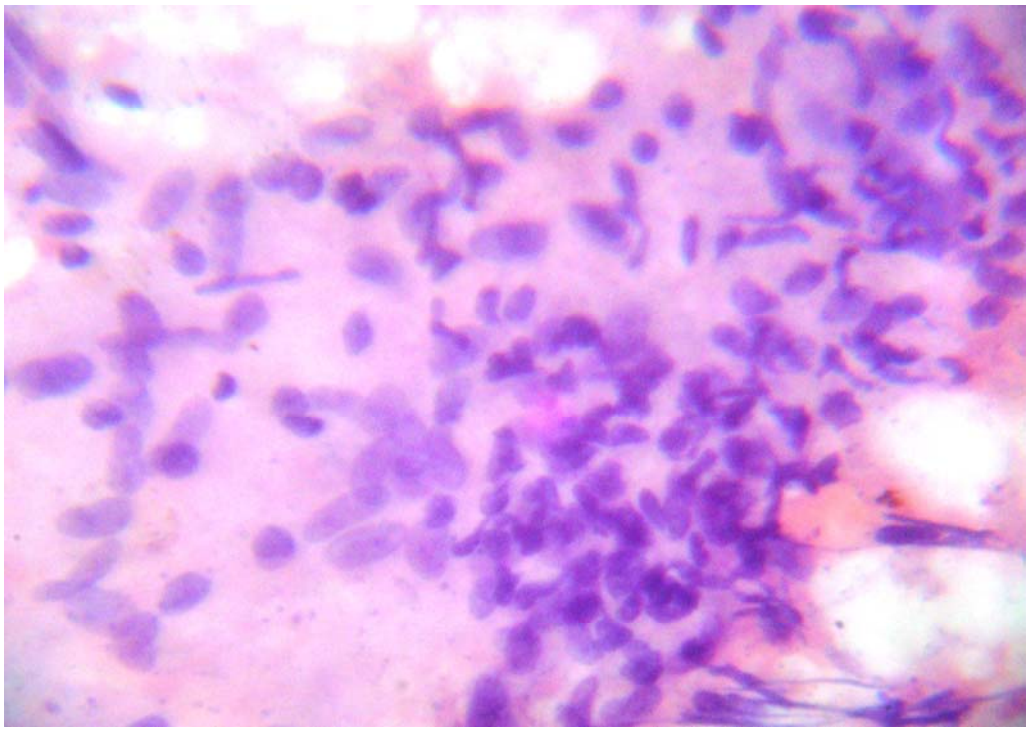


Fig-2- 40X- showing epithelioid cells. (H&E)

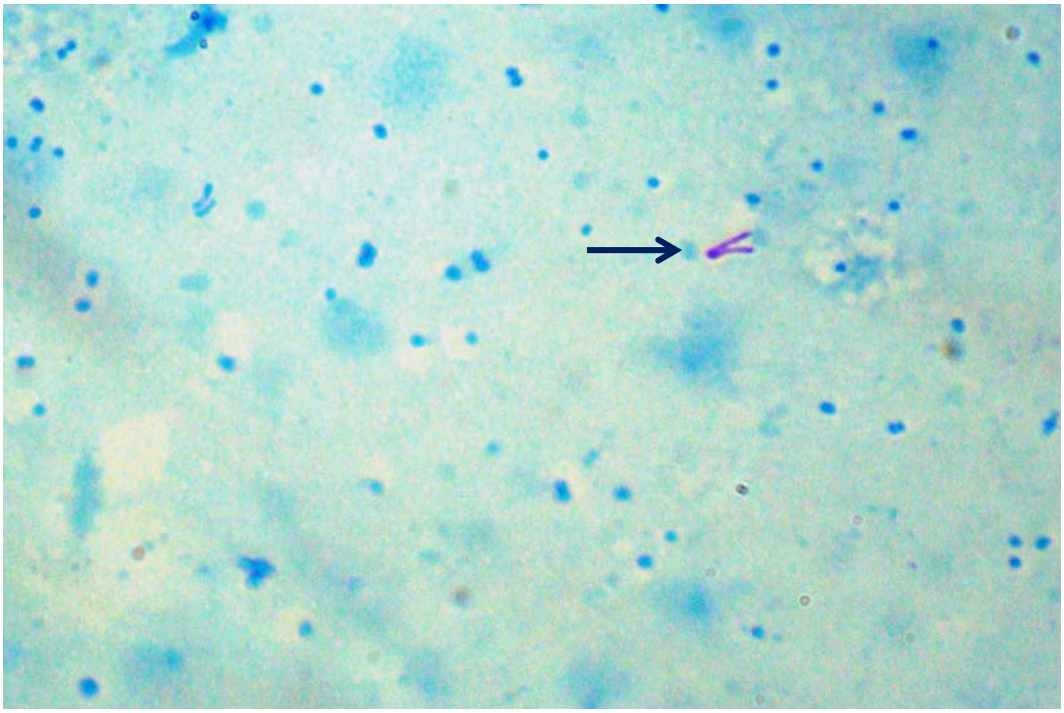


Fig-3- AFB seen as pink rods under oil immersion (ZN stain)

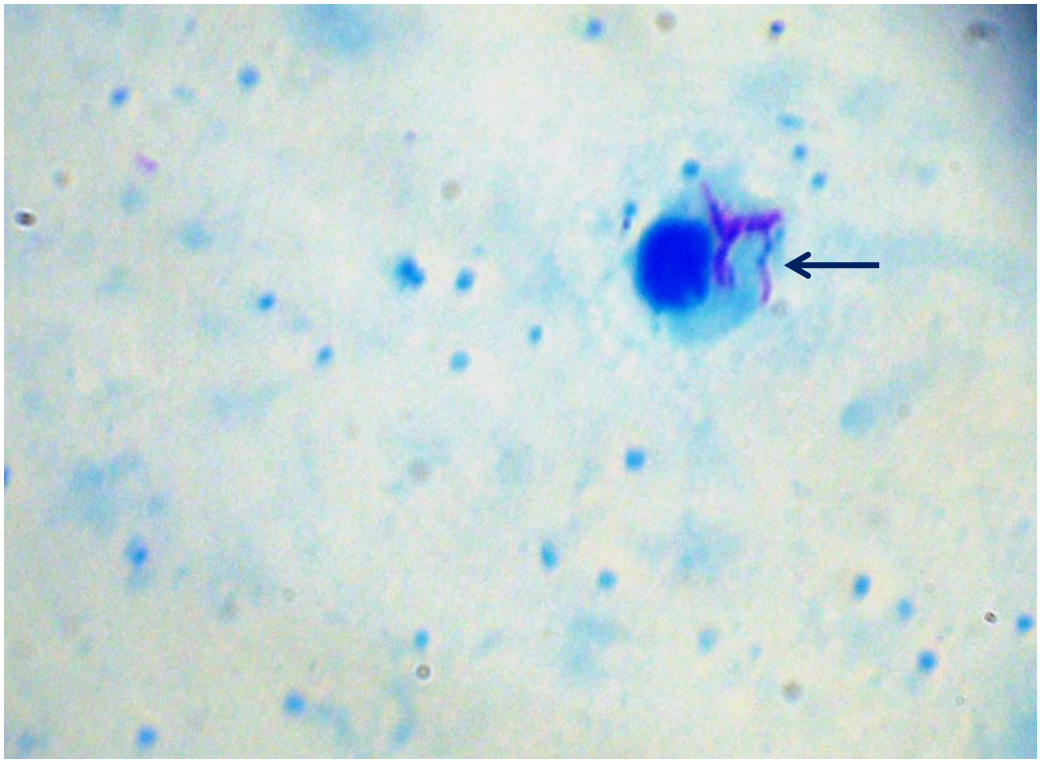


Fig-4- AFB under oil immersion (ZN stain)

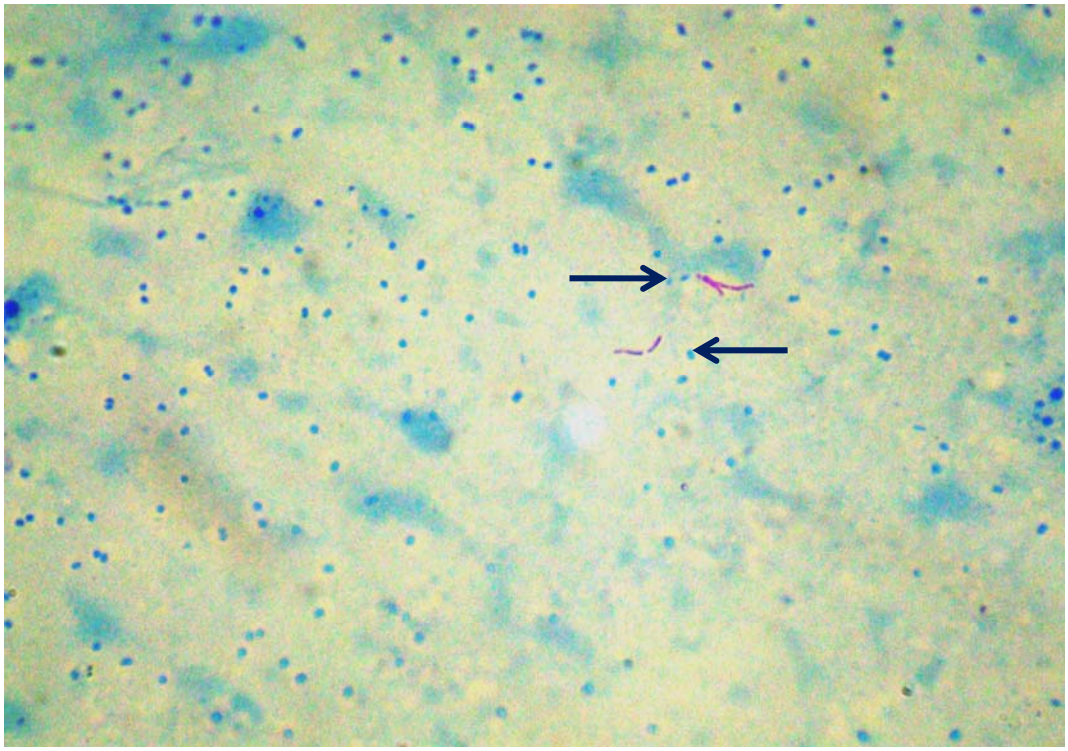


Fig-5- pink slender rod shaped AFB under 100X (ZN stain)

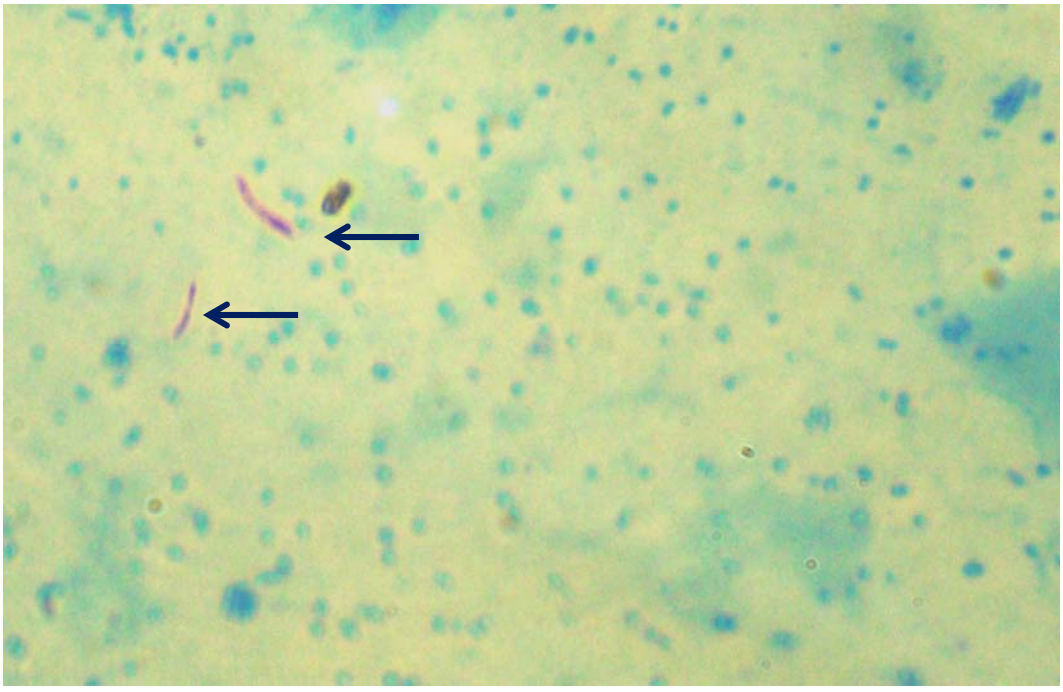


Fig-6- slender rod shaped AFB 100X (ZN stain)

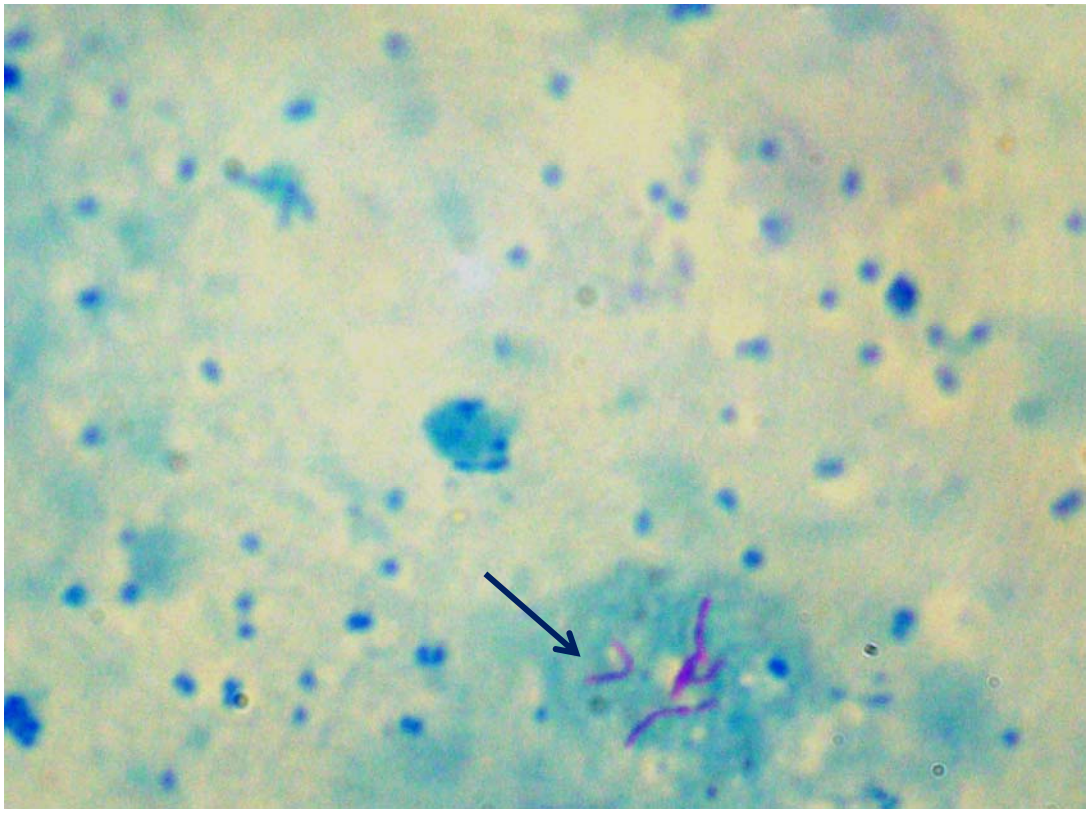


Fig-7-AFB seen as pink rods 100X (ZN stain)

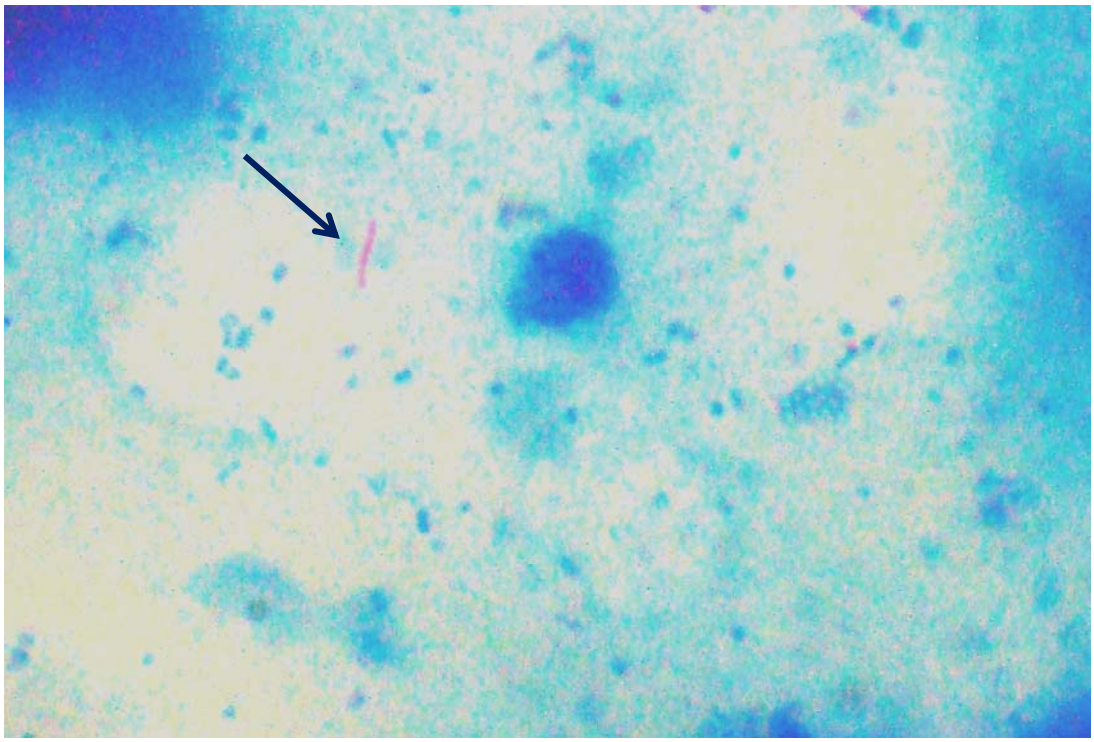


Fig-8- AFB seen under oil immersion (ZN stain)

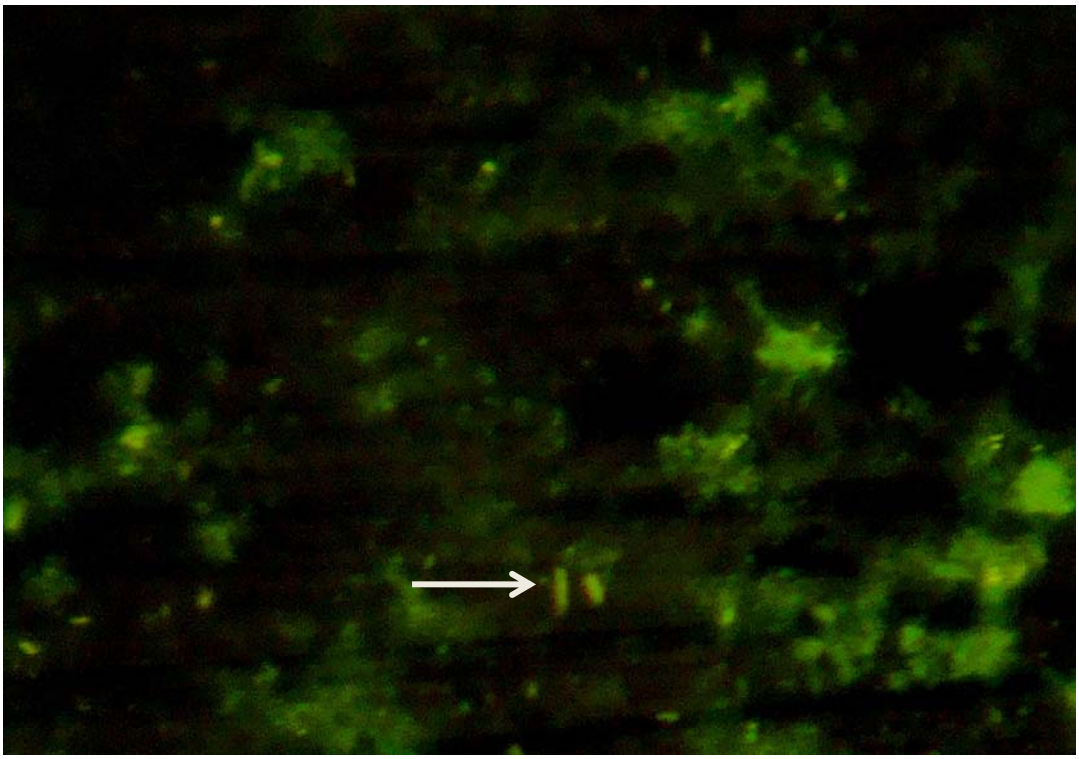


Fig-9 – AFB under fluorescence microscope 20X (Auramine O stain)

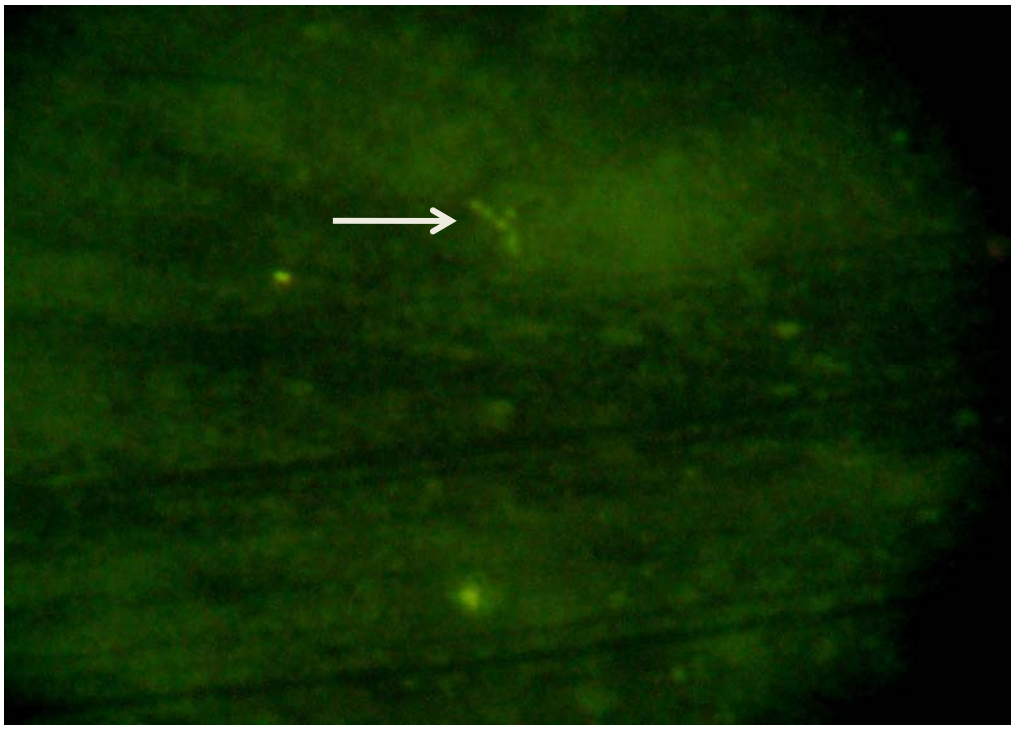


Fig-10- AFB under 20X fluorescence microscope (Auramine O)

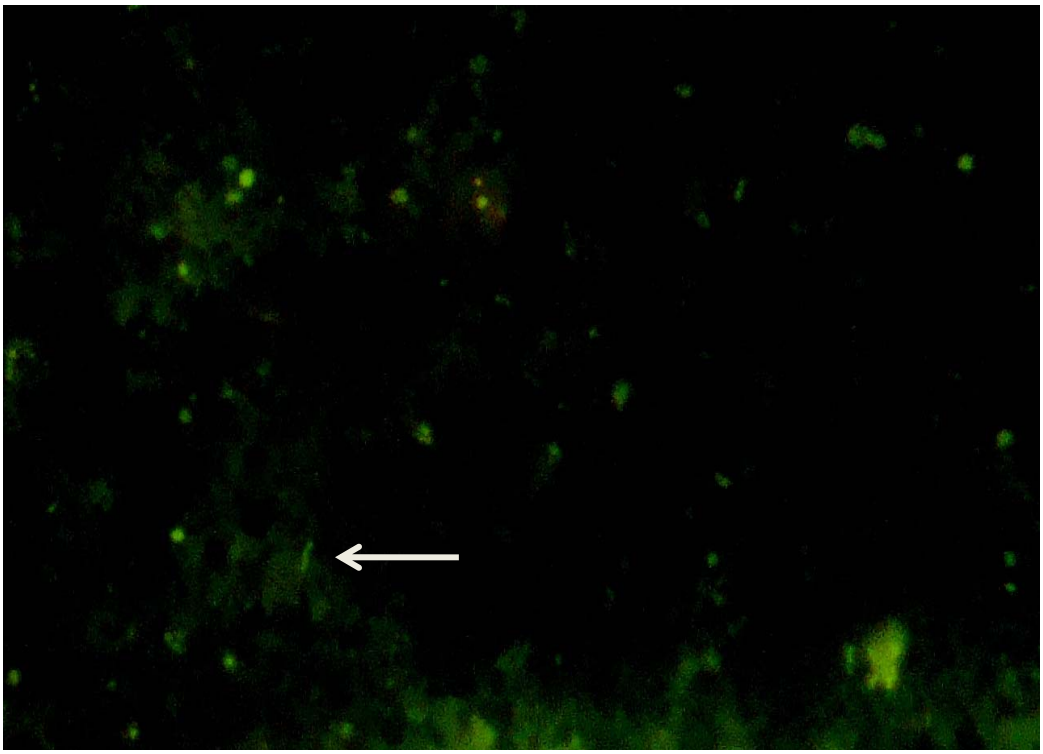


Fig-11- AFB under 20X fluorescence microscope (Auramine O stain)

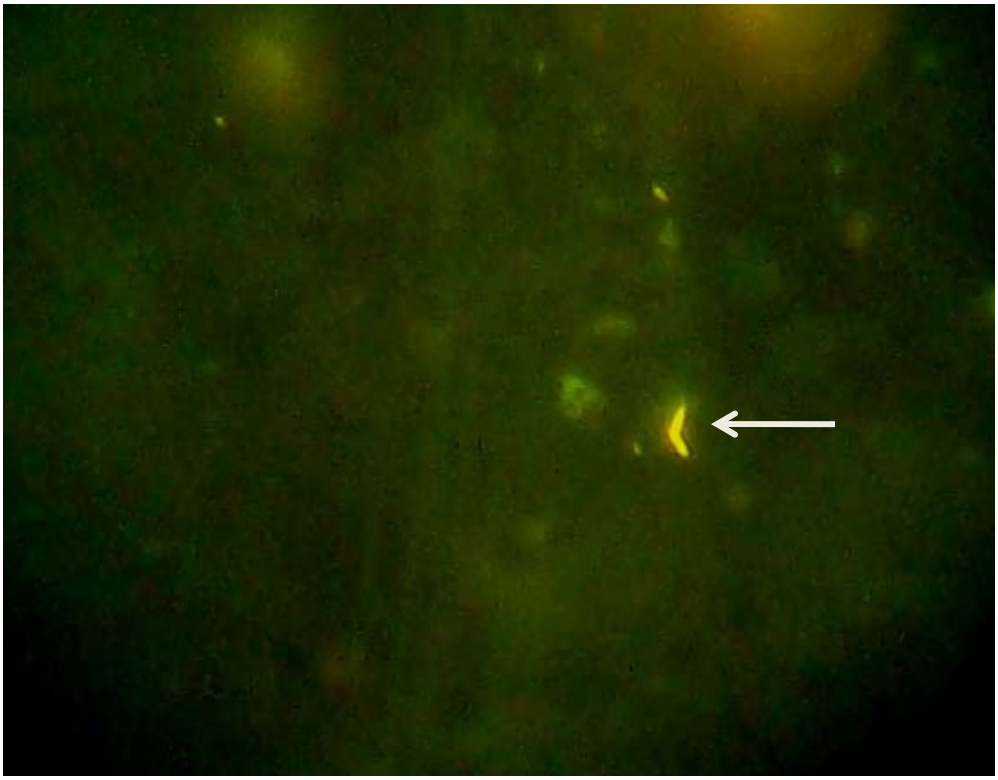


Fig-12- AFB seen as yellowish rods under oil immersion (Auramine O)

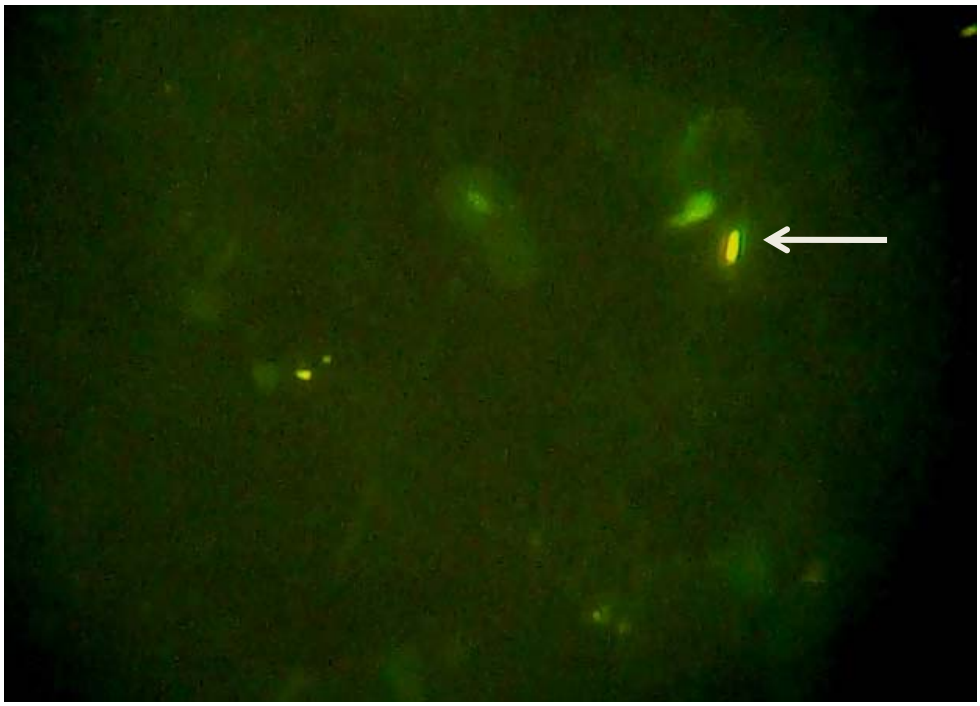


Fig-13- yellow rod shaped AFB seen under oil immersion (Auramine O)

DISCUSSION

DISCUSSION

Tuberculosis (TB) continues to be a major health problem in developing countries. There are nearly 9 million new cases and 2 million deaths from tuberculosis world wide every year[41]. Lymphadenitis is one of the most common extrapulmonary manifestation of tuberculosis. Tuberculous lymphadenitis is a local manifestation of the systemic disease[42].

The clinical parameters for the diagnosis of TB in lymph nodes are neither specific nor do their absence exclude TB involvement[43]. Fine-needle aspiration cytology (FNAC) of lymph nodes in TB has varied cytomorphological features. However, the conventional Ziehl–Neelsen (ZN) method for acid-fast bacilli (AFB) plays a key role in the diagnosis and also for the monitoring of treatment in TB. Its major disadvantage is its low sensitivity ranging from 20% to 43%[44]. Mycobacterial culture is more sensitive and specific and also the reference method for the detection of tubercle bacilli but it is time consuming and requires specialized safety procedures in laboratories. Serological techniques have the disadvantage of lack of sensitivity and specificity[45]. Newer molecular techniques such as Polymerase chain reaction (PCR), although rapid, are costly to be routinely used in developing countries where most TB cases occur[46]. Hence, a

method for the identification of AFB which is more sensitive than the ZN method is required for the early detection of TB.

In this study, aspiration cytology smears of patients with suspected tuberculous lymphadenitis were taken. The demographic data of the cases were also recorded and analysed.Only the smears which were diagnosed as granulomatous lymphadenitis on routine cytology by H&E were considered for the study.Out of the 212 cases registered,112 cases were rejected because the H&E showed either reactive lymphadenitis,metastatic deposits or lymphoproliferative disorder.Only those cases diagnosed as granulomatous lesions were included in this study.A total of 100 granulomatous lesions were taken for Ziehl Neelsen and phenolic auramine staining. The rate of detection of AFB by both the stains were compared. Also the routine cytologic features were compared with rate of detection of AFB.

Mycobacterial lymphadenitis may affect patients of any age. There is a female predominance in most of the studies [47]. In this study, the age range of tuberculous lymphadenitis was found to be 3 to 70 years and there was a female predominance.(63.15%).(Table-1).

Patients generally present with low grade fever, weight loss and fatigue and less frequently with night sweats.[48]. In this study, the most common symptom was found to be fever(84.21%). Other symptoms were weight loss, night sweats and cough.(Table-2).

Tuberculous lymphadenitis most frequently involves the cervical lymph nodes followed in frequency by mediastinal, axillary, mesenteric, hepatic portal, perihepatic and inguinal lymph nodes[49]. In this study,the most common lymphnode group involved was cervical group(57.9%). Other lymphnodes found to be involved were supraclavicular and inguinal nodes.(Table-3).

Multiplicity, matting and caseation are three important findings of tuberculous lymphadenitis[50]. In this study, most of the cases showed unilateral involvement of nodes(89.4%). Involved lymphnodes were found to be matted(68.4%). Abcess formation was commonly seen(78.9%).(Table-4).

Associated chest lesions as seen on chest radiography are very common in children but less common in adults, evident nearly in 15% cases[51]. In this study, X – ray chest showed no significant findings in most of the cases(52.63%). Findings like pneumonitis, fibrosis and bronchiectasis were found in few cases.(Table-5).

The aspirates from lymphnodes are usually diagnosed as tuberculaous lymphadenopathy based on the presence of either epithelioid cell granuloma with or without langhan’s giant cells, with necrosis in a milieu of parent lymphoid cells or epithelioid cells granuloma without necrosis and caseation or only necrotic material consisting of diffuse granular debris[52]. In this study, epithelioid cells were found in fine needle aspiration smears of all the

cases of tuberculous lymphadenitis(100%). Giant cells(42.1%) and caseation necrosis(84.2%) were also seen in.(Table-6).(Figs-1-2).

Auramine stain was positive when caseation (7/8) and epitheliod cells (8/8) were present in the smears.(Table-8).

Among 100 cases of granulomatous lymphadenitis, acid fast bacilli was detected in 11%(11/100 cases) by Ziehl Neelsen (Figs-3-8) and in 19%(19/100) cases by Auramine stain.(Table-8) (Figs-9-14). All Ziehl Neelsen positive cases were also positive for Auramine stain. Auramine stain also showed positivity in 8 cases in which Ziehl Neelsen was negative. This shows that the rate of detection of AFB by Auramine stain(19%) was more than conventional Ziehl Neelsen stain(8%). Ziehl Neelsen stain failed to detect AFB in 89/100 cases(Table-9).flourescent stain was negative in 81/100 cases. Thus fluorescent method was found to be more sensitive than the conventional Ziehl Neelsen method in this study.

The evaluation of both the stains are as follows;

Evaluation of fluorescent stain

sensitivity = true positive = 11/11x100 = 100%

specificity = true negative = 81/89x100 = 91%

positive predictive value = 11/19x100 = 58%

negative predictive value = 81/81x100 = 100%

Evaluation of Ziehl-Neelsen stain

sensitivity = true positive = 11/19x100 = 58%

specificity = true negative = 81/81x100=100%

positive predictive value = 11/11x100 = 100%

negative predictive value = 81/89x100 = 91%

The comparative results of the two staining techniques of this study are tabulated in Table-10. The sensitivity of fluorescent stain was found to be higher(100%) than ZN(58%). The overall efficacy of fluorescent stain in the detection of AFB was 19%(19/100 cases) which is 8% more than the conventional ZN-11%(11/100 cases) in this study.(Table-10).

This is in accordance with a study conducted by Vamseedhar Annam et al. The study was conducted in lymphnode aspirates. Out of 108 aspirates, 102 were studied and remaining 6 were excluded from the study due to diagnosis of malignancy in 4.04% (4/6) and inadequate aspiration in 2.02% (2/6). Among the 102 aspirates, 44.11% (45/102) were positive for AFB on the conventional ZN method, 58.9% (60/102) were indicative of TB on cytology, while the smear positive increased to 81.37% (83/102) on the modified fluorescent method. They concluded that the bacillary positivity

rates were higher in the modified fluorescent method than in the ZN method. Hence, the fluorescent method can be an adjuvant when used with routine cytology for the identification of AFB[53].

Also in a study done by Laifangbam S et al[26], Out of 102 patients, 44.1%, 71.6% and 70% were found positive by ZN, AO and culture respectively. AO was found to be superior to ZN on several aspects. AO was also able to detect more pauci-bacillary cases than ZN. There was more agreement between culture and fuorescence microscopy (95.1%) than with ZN microscopy (69.6%). The percentage of false negative by AO staining was only 2.78% which was in sharp contrast to that of ZN (40.27%). They also concluded that the better case detection rates of AO over ZN were comparable to those found by several studies. Since screening was done under lower power of magnification (400x), fluorescence microscopy has been found to be less time consuming as compared to ZN method (1000x) in the diagnosis of tuberculosis. The tubercle bacilli stood out as bright objects against a dark background in fluorescence microscopy which makes them easily identifiable hence causing less eye-strain. The efficacy of fluorescence microscopy proved to be much higher than conventional light microscopy[54].

Higher positive yield by fluorescent microscopy was noted which suggest greater sensitivity of the technique. Expectedly, due to the lower

magnification used to scan fluorochrome stained smears, it was able to view a much larger area of the smear. In addition, fluorescing green- yellow bacillus in a dark background is easier and less strenuous for the eye to detect.

The findings of this study showed that Auramine O fluorescent stain is a more reliable and a sensitive technique for AFB detection especially in laboratories with a heavy work load as it is more sensitive and accurate than the conventional ZN stain.

SUMMARY AND

CONCLUSION

SUMMARY AND CONCLUSION

The commonest manifestation of extra pulmonary tuberculosis is lymphadenitis. In clinically suspected cases of TB lymphadenitis, FNAC of lymph node is a reliable, safe, simple and valuable diagnostic tool in identifying the cause of lymphadenitis. Identifying AFB in cytological smears will help to give a definitive diagnosis. A definitive diagnosis will help in initiating early ATT therapy to the patients. Routine H&E stains fail to identify AFB in a majority of cases. Ziehl-Neelsen is the gold standard method used prevalently for the detection of AFB. Due to its low sensitivity and higher percentage of false negativity we need to employ a more sensitive screening test. Fluorecent stains have been found to be more sensitive than the conventional ZN stain. This study was undertaken to compare and evaluate the relative efficacy of ZN and fluorescent stains in TB lymph node FNA samples taking ZN as the gold standard.

Out of the 100 granulomatous lesions reported in H&E examination, ZN was found to be positive in 11/100 cases. AO was also positive in all the 11 ZN positive cases. In addition AO was found to be positive in 8/89 ZN negative cases bringing the total positivity for AO to 19/100 cases.

The study found fluorescent stain to be more sensitive than ZN stains in identifying the AFB. The method of staining was easy and quick. The bacilli could be identified easily under lower magnification and the

number of fields screened were also less when compared with ZN stain. The false negativity was significantly more for ZN stain due to its low sensitivity and hence it is a less sensitive test screening test when compared with fluorescent stains.

The fluorescence due to AFB can be confirmed by restaining with ZN stain and try to differentiate the non specific fluorescence by identifying AFB morphology if in doubt .The major restraint in using flouresencent stains routinely is the cost factor. The cost of flourecence microscope is high. The working cost per slide is also higher when compared with ZN stain.

Fluorescence microscopy is a useful, rapid, and reliable and a sensitive tool for the examination of specimens for AFB. It helps in rapid identification of AFB in smears reported as granulomatous lesion in H&E. As the cause of granulomatous lesions are varied a definitive diagnosis will help in early, appropriate treatment intervention which will benefit the patient and the community as a whole. Hence it can be readily employed as an adjuvant to the routine H&E stain. It should be seriously considered for supplementary use in laboratories that handle large numbers of specimens. Initial capital investment costs, maintenance and fluctuation in electric current will never the less preclude its widespread use at the peripheral level(provincial or regional) in many low income countries. But it should quite clearly be available in every national reference laboratory and teaching institutions that handles large numbers of routine specimens.

MASTER CHART

S. No	CYTOLOGY NO	AGE	SEX	FEVER	WEIGHT LOSS	NIGHT SWEATS	COUGH	H/O ATT/ART	LYMPH NODE							SPLENO-MEGALY	HEPATO-MEGALY	OTHER CLINICAL FINDINGS	IMAGING	MANT-OUX	SPUTUM AFB	BLOOD INVESTIGATIONS				H&E			Z-N	GRADE	AO	GRADE
									SITE	SIZE (cm)	SINGLE/ MATTED	ABCESS	UL/BL	SINUS	STAGE (J&C)							LYM %	ABS. LYM.C	ESR (mm)	HIV	GIANT CELL	EPITHEL-IOID CELL	CASE-ATION				
1	4358/11	30	F	Y	Y	N	N	N	CERVICAL	2X1	MATTED	Y	UL	N	2	N	N	N	NL	P	NEG	62	8850	43/56	N	Y	Y	Y	N		N	
2	4363/11	6	F	Y	Y	Y	Y	N	CERVICAL	2.5X1.5	MATTED	N	BL	N	2	N	N	N	BASAL PNEUMONITIS	P	NEG	67	9240	27/42	N	Y	Y	N	N		N	
3	4375/11	39	M	N	N	N	N	N	CERVICAL	3.5X1.5	SINGLE	N	UL	N	2	N	N	N	BASAL PNEUMONITIS	INTM	NEG	58	6250	46/58	N	N	Y	N	Y	2+	Y	1+
4	4386/11	18	F	Y	Y	N	N	N	CERVICAL	3X2	MATTED	Y	UL	N	3	N	N	N	NL	N	NEG	60	8450	36/40	N	N	Y	Y	N		N	
5	4391/11	17	M	Y	Y	N	Y	N	CERVICAL	2X2	MATTED	N	UL	N	2	N	N	N	NL	P	NEG	68	9800	32/40	N	Y	Y	N	N		N	
6	4392/11	16	F	Y	Y	N	Y	N	CERVICAL	1X1	SINGLE	Y	UL	N	3	N	N	N	PNEUMONITIS	P	NEG	55	6450	28/36	N	N	Y	Y	N		Y	1+
7	4408/11	35	M	N	Y	Y	N	N	CERVICAL	3X2.5	MATTED	Y	UL	N	3	N	N	N	HAZINESS	P	NEG	69	8850	33/46	N	Y	Y	Y	N		N	
8	4409/11	25	F	N	N	N	N	Y	CERVICAL	1X1	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	65	9850	29/36	N	N	Y	N	N		N	
9	4414/11	5	M	Y	N	N	Y	N	SUBMANDIBULAR, CERVICAL	1X1,2X1	MATTED	N	BL	N	2	N	N	N	NL	N	NEG	54	6150	22/32	N	N	Y	N	N		N	
10	4420/11	25	F	N	Y	N	N	N	CERVICAL	2X2	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	52	5950	24/32	N	Y	Y	N	N		N	
11	4427/11	42	M	N	Y	N	N	N	AXILLARY	1X1	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	55	6460	28/36	N	Y	Y	Y	N		N	
12	4432/11	48	F	N	Y	N	N	N	CERVICAL	3X3	MATTED	Y	UL	N	3	N	N	N	NL	P	NEG	57	6125	26/34	N	N	Y	Y	N		N	
13	4434/11	19	F	Y	N	Y	Y	N	CERVICAL	1X1	SINGLE	N	UL	N	2	N	N	N	BRONCHIECTASIS	P	NEG	64	9850	24/32	N	Y	Y	N	N		N	
14	4463/11	15	F	Y	Y	N	Y	N	CERVICAL	1X1	SINGLE	Y	UL	N	3	N	N	N	PNEUMONITIS	P	NEG	61	7450	26/38	N	N	Y	Y	N		N	
15	4467/11	3	M	Y	N	N	N	N	INGUINAL	1X0.5	MATTED	Y	UL	N	3	N	N	N	NL	P	NEG	74	14600	42/56	N	N	Y	Y	N		Y	2+
16	4471/11	45	F	Y	Y	N	N	N	CERVICAL	3X2	SINGLE	N	UL	N	2	N	N	N	PNEUMONITIS	P	NEG	68	9400	46/58	N	Y	Y	N	N		N	
17	4482/11	45	F	Y	Y	N	Y	N	CERVICAL	1.5X1	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	65	7400	36/52	N	Y	Y	N	N		N	
18	4484/11	28	F	Y	Y	N	N	N	CERVICAL	2X1	SINGLE	N	UL	N	2	N	N	N	PNEUMONITIS	INTM	NEG	62	8400	38/46	N	Y	Y	N	N		N	
19	4492/11	10	M	Y	N	N	Y	N	CERVICAL	3X2	MATTED	Y	UL	N	3	N	N	N	NL	P	NEG	69	9800	28/36	N	Y	Y	Y	Y	1+	Y	1+
20	4500/11	32	F	Y	Y	N	Y	N	AXILLARY	2X1	SINGLE	N	UL	N	2	N	N	N	BRONCHIECTASIS	P	NEG	72	10800	38/46	N	N	Y	Y	N		N	
21	4507/11	45	M	N	Y	N	N	N	CERVICAL	1X0.5	SINGLE	N	UL	N	2	N	N	N	NL	INTM	NEG	45	4200	24/32	N	N	Y	N	N		N	
22	4508/11	70	F	Y	Y	N	N	N	INGUINAL	2X2	MATTED	Y	BL	N	3	N	N	N	NL	P	NEG	62	9400	28/36	N	Y	Y	Y	N		Y	1+
23	4511/11	17	M	Y	Y	N	N	N	CERVICAL	0.5X0.5	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	48	4800	24/32	N	N	Y	N	N		N	
24	4518/11	35	F	N	Y	N	N	N	CERVICAL	2X1	MATTED	N	BL	N	2	N	N	N	NL	N	NEG	62	7800	36/42	N	N	Y	N	N		N	
25	4522/11	13	F	N	N	N	Y	N	HYPOCHONDRIAL REGION, ANT. ABDOMINAL WALL	1.5X0.5		N	UL	N		N	Y	N	HILAR NODES+	P	NEG	68	8200	42/56	N	N	Y	Y	N		N	
26	4530/11	55	M	Y	Y	Y	Y	N	SUPRACLAVICULAR	1X1.5	SINGLE	Y	UL	N	3	N	N	N	PNEUMONITIS	P	NEG	48	6200	24/36	N	Y	Y	N	N		N	
27	4533/11	46	F	N	N	N	Y	N	CERVICAL	1X1	SINGLE	N	BL	N	1	N	N	N	NL	P	NEG	40	4800	28/42	N	N	Y	N	N		N	
28	4537/11	40	M	Y	Y	Y	Y	H/OART	SUPRACLAVICULAR	3X2	MATTED	Y	UL	N	3	N	Y	N	CAVITY	N	NEG	18	720	32/42	P	N	Y	Y	N		N	
29	4572/11	29	F	Y	Y	N	N	N	CERVICAL	1.5X1	MATTED	N	UL	N	2	N	N	N	NL	INTM	NEG	58	2400	40/52	N	Y	Y	N	N		N	
30	4580/11	29	F	Y	Y	N	Y	N	CERVICAL	0.5X0.5	MULTIPLE/DISCRETE	N	UL	N	2	N	N	N	NL	P	NEG	52	2400	36/42	N	Y	Y	N	N		N	
31	4583/11	24	F	Y	Y	N	Y	N	CERVICAL	1X0.5	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	21	840	30/36	P	N	Y	Y	N		N	
32	4622/11	15	M	Y	Y	Y	N	N	INGUINAL	2X2	SINGLE	N	BL	Y	4	N	N	N	NL	P	NEG	68	2400	42/56	N	Y	Y	Y	Y	1+	Y	1+
33	4644/11	38	F	Y	Y	N	N	H/OATT	SUPRACLAVICULAR	2X1	MATTED	Y	UL	N	3	N	N	N	BRONCHIECTASIS	P	NEG	52	2400	38/46	N	N	Y	Y	N		Y	2+
34	4649/11	28	F	Y	N	N	N	N	SUPRACLAVICULAR	2X1	MATTED	Y	UL	N	3	N	N	N	NL	P	NEG	56	2400	18/24	N	N	Y	Y	Y	1+	Y	1+
35	4665/11	49	M	Y	N	N	N	H/OATT	INGUINAL	3X2	MATTED	Y	BL	Y	3	N	N	N	NL	P	NEG	58	2400	36/48	N	Y	Y	Y	N		N	
36	4672/11	11	F	N	N	N	N	N	CERVICAL	1X0.5	MATTED	N	UL	N	2	N	N	N	NL	P	NEG	42	2400	22/28	N	Y	Y	N	N		N	
37	4732/11	28	M	N	N	N	N	N	SUPRACLAVICULAR	3X2	MATTED	Y	UL	N	3	N	N	N	PNEUMONITIS	P	NEG	48	2400	28/32	N	N	Y	Y	N		N	
38	4736/11	20	F	N	Y	N	N	N	CERVICAL	1X1	SINGLE	N	UL	N	2	N	N	N	NL	INTM	NEG	44	2400	20/32	N	N	N	Y	N		N	
39	4779/11	9	F	Y	N	N	N	N	CERVICAL	2X1	MATTED	Y	UL	N	3	N	Y	N	NL	P	NEG	52	2400	22/28	N	Y	Y	N	N		N	
40	4777/11	27	F	N	N	N	N	N	CERVICAL	1X1	SINGLE	N	UL	N	2	N	Y	N	NL	INTM	NEG	42	2400	20/26	N	N	Y	N	N		N	
41	4787/11	36	F	Y	Y	N	N	H/OATT	CERVICAL	2X1	MATTED	Y	UL	N	3	N	N	N	PNEUMONITIS	P	NEG	68	2400	38/42	N	Y	Y	Y	Y	1+	Y	1+
42	4789/11	8	M	Y	N	N	N	N	SUPRACLAVICULAR	1X0.5	MULTIPLE/DISCRETE	N	UL	N	2	N	N	N	NL	P	NEG	74	2400	40/52	N	N	Y	Y	N		N	
43	4791/11	19	F	N	N	N	N	N	CERVICAL	3X2	SINGLE	Y	UL	N	3	N	N	N	NL	P	NEG	72	2400	46/52	N	N	Y	Y	N		N	
44	4798/11	8	F	Y	N	N	N	N	CERVICAL	2X1.5	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	40	2400	24/32	N	N	Y	N	N		N	

S. No	CYTOLOGY NO	AGE	SEX	FEVER	WEIGHT LOSS	NIGHT SWEATS	COUGH	H/O ATT/ART	LYMPH NODE							SPLENO-MEGALY	HEPATO-MEGALY	OTHER CLINICAL FINDINGS	IMAGING	MANT-OUX	SPUTUM AFB	BLOOD INVESTIGATIONS				H&E			Z-N	GRADE	AO	GRADE
									SITE	SIZE (cm)	SINGLE/ MATTED	ABCESS	UL/BL	SINUS	STAGE (J&C)							LYM %	ABS. LYM.C	ESR (mm)	HIV	GIANT CELL	EPITHEL-IOID CELL	CASE-ATION				
45	4804/11	6	M	Y	N	N	Y	N	CERVICAL	2X2	MATTED	Y	UL	N	3	N	N	N	PNEUMONITIS	P	NEG	74	2400	36/46	N	N	Y	Y	N		N	
46	4817/11	53	F	Y	Y	N	Y	H/OATT	SUBMANDIBULAR	1X1	SINGLE	N	UL	Y	4	N	N	N	CONSOLIDATION	P	NEG	68	2400	48/52	N	Y	Y	Y	N		N	
47	4832/11	54	M	Y	Y	N	N	N	CERVICAL	1X1	MULTIPLE	N	UL	N	2	N	Y	N	HILAR NODES+	P	NEG	76	2400	48/56	N	N	Y	N	N		N	
48	4848/11	5	M	Y	N	N	N	N	CERVICAL	2X2	MATTED	Y	UL	N	3	N	N	N	NL	P	NEG	78	2400	38/48	N	N	Y	Y	N		N	
49	4862/11	24	F	Y	Y	N	N	N	CERVICAL	2X2	SINGLE	N	UL	N	2	N	N	N	NL	INTM	NEG	42	2400	24/32	N	N	Y	N	N		N	
50	4872/11	18	F	Y	Y	N	N	Y	CERVICAL	3X2	MATTED	Y	UL	N	3	N	N	N	FIBROSIS	P	NEG	62	2400	40/52	N	N	Y	Y	Y	2+	Y	2+
51	4891/11	35	M	N	Y	Y	N	N	CERVICAL	3X2.5	MATTED	Y	UL	N	3	N	N	N	HAZINESS	P	NEG	69	2400	33/46	N	Y	Y	Y	N		N	
52	4996/11	25	F	N	N	N	N	Y	CERVICAL	1X1	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	65	2400	29/36	N	N	Y	N	N		N	
53	4997/11	5	M	Y	N	N	Y	N	SUBMANDIBULAR, CERVICAL	1X1,2X1	MATTED	N	BL	N	2	N	N	N	NL	N	NEG	54	2400	22/32	N	N	Y	N	N		N	
54	4998/11	25	F	N	Y	N	N	N	CERVICAL	2X2	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	52	2400	24/32	N	Y	Y	N	N		N	
55	5028/11	42	M	N	Y	N	N	N	AXILLARY	1X1	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	55	2400	28/36	N	Y	Y	Y	N		N	
56	5085/11	38	F	Y	Y	N	N	H/OATT	SUPRACLAVICULAR	2X1	MATTED	Y	UL	N	3	N	N	N	BRONCHIECTASIS	P	NEG	52	2400	38/46	N	N	Y	Y	N		Y	1+
57	5112/11	28	F	Y	N	N	N	N	SUPRACLAVICULAR	2X1	MATTED	Y	UL	N	3	N	N	N	NL	P	NEG	56	2400	18/24	N	N	Y	Y	Y	2+	Y	2+
58	5116/11	49	M	Y	N	N	N	H/OATT	INGUINAL	3X2	MATTED	Y	BL	Y	3	N	N	N	NL	P	NEG	58	2400	36/48	N	Y	Y	Y	N		N	
59	5129/11	11	F	N	N	N	N	N	CERVICAL	1X0.5	MATTED	N	UL	N	2	N	N	N	NL	P	NEG	42	2400	22/28	N	Y	Y	N	N		N	
60	5131/11	28	M	N	N	N	N	N	SUPRACLAVICULAR	3X2	MATTED	Y	UL	N	3	N	N	N	PNEUMONITIS	P	NEG	48	2400	28/32	N	N	Y	Y	N		N	
61	5134/11	20	F	N	Y	N	N	N	CERVICAL	1X1	SINGLE	N	UL	N	2	N	N	N	NL	INTM	NEG	44	2400	20/32	N	N	N	Y	N		N	
62	5150/11	9	F	Y	N	N	N	N	CERVICAL	2X1	MATTED	Y	UL	N	3	N	Y	N	NL	P	NEG	52	2400	22/28	N	Y	Y	N	N		N	
63	5170/11	27	F	N	N	N	N	N	CERVICAL	1X1	SINGLE	N	UL	N	2	N	Y	N	NL	INTM	NEG	42	2400	20/26	N	N	Y	N	N		N	
64	5175/11	36	F	Y	Y	N	N	H/OATT	CERVICAL	2X1	MATTED	Y	UL	N	3	N	N	N	PNEUMONITIS	P	NEG	68	2400	38/42	N	Y	Y	Y	Y	2+	Y	1+
65	5183/11	8	M	Y	N	N	N	N	SUPRACLAVICULAR	1X0.5	MULTIPLE/DISCRETE	N	UL	N	2	N	N	N	NL	P	NEG	74	2400	40/52	N	N	Y	Y	N		N	
66	5188/11	19	F	N	N	N	N	N	CERVICAL	3X2	SINGLE	Y	UL	N	3	N	N	N	NL	P	NEG	72	2400	46/52	N	N	Y	Y	N		N	
67	5189/11	8	F	Y	N	N	N	N	CERVICAL	2X1.5	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	40	2400	24/32	N	N	Y	N	N		N	
68	5195/11	6	M	Y	N	N	Y	N	CERVICAL	2X2	MATTED	Y	UL	N	3	N	N	N	PNEUMONITIS	P	NEG	74	2400	36/46	N	N	Y	Y	N		N	
69	5207/11	53	F	Y	Y	N	Y	H/OATT	SUBMANDIBULAR	1X1	SINGLE	N	UL	Y	4	N	N	N	CONSOLIDATION	P	NEG	68	2400	48/52	N	Y	Y	Y	N		N	
70	5209/11	54	M	Y	Y	N	N	N	CERVICAL	1X1	MULTIPLE	N	UL	N	2	N	Y	N	HILAR NODES+	P	NEG	76	2400	48/56	N	N	Y	N	N		N	
71	5211/11	5	M	Y	N	N	N	N	CERVICAL	2X2	MATTED	Y	UL	N	3	N	N	N	NL	P	NEG	78	2400	38/48	N	N	Y	Y	N		N	
72	5216/11	24	F	Y	Y	N	N	N	CERVICAL	2X2	SINGLE	N	UL	N	2	N	N	N	NL	INTM	NEG	42	2400	24/32	N	N	Y	N	N		N	
73	5218/11	30	F	Y	Y	N	N	N	CERVICAL	2X1	MATTED	Y	UL	N	2	N	N	N	NL	P	NEG	62	2400	43/56	N	Y	Y	Y	N		N	
74	5220/11	6	F	Y	Y	Y	Y	N	CERVICAL	2.5X1.5	MATTED	N	BL	N	2	N	N	N	BASAL PNEUMONITIS	P	NEG	67	2400	27/42	N	Y	Y	N	N		N	
75	5237/11	39	M	N	N	N	N	N	CERVICAL	3.5X1.5	SINGLE	N	UL	N	2	N	N	N	BASAL PNEUMONITIS	INTM	NEG	58	2400	46/58	N	N	Y	N	Y	1+	Y	1+
76	5238/11	18	F	Y	Y	N	N	N	CERVICAL	3X2	MATTED	Y	UL	N	3	N	N	N	NL	N	NEG	60	2400	36/40	N	N	Y	Y	N		N	
77	5242/11	17	M	Y	Y	N	Y	N	CERVICAL	2X2	MATTED	N	UL	N	2	N	N	N	NL	P	NEG	68	2400	32/40	N	Y	Y	N	N		N	
78	5296/11	16	F	Y	Y	N	Y	N	CERVICAL	1X1	SINGLE	Y	UL	N	3	N	N	N	PNEUMONITIS	P	NEG	55	2400	28/36	N	N	Y	Y	N		Y	2+
79	5303/11	35	M	N	Y	Y	N	N	CERVICAL	3X2.5	MATTED	Y	UL	N	3	N	N	N	HAZINESS	P	NEG	69	2400	33/46	N	Y	Y	Y	N		N	
80	5306/11	25	F	N	N	N	N	Y	CERVICAL	1X1	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	65	2400	29/36	N	N	Y	N	N		N	
81	5310/11	5	M	Y	N	N	Y	N	SUBMANDIBULAR, CERVICAL	1X1,2X1	MATTED	N	BL	N	2	N	N	N	NL	N	NEG	54	2400	22/32	N	N	Y	N	N		N	
82	5313/11	25	F	N	Y	N	N	N	CERVICAL	2X2	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	52	2400	24/32	N	Y	Y	N	N		Y	1+
83	5316/11	42	M	N	Y	N	N	N	AXILLARY	1X1	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	55	2400	28/36	N	Y	Y	Y	N		N	
84	5317/11	48	F	N	Y	N	N	N	CERVICAL	3X3	MATTED	Y	UL	N	3	N	N	N	NL	P	NEG	57	2400	26/34	N	N	Y	Y	N		N	
85	5321/11	15	F	Y	Y	N	Y	N	CERVICAL	1X1	SINGLE	Y	UL	N	3	N	N	N	PNEUMONITIS	P	NEG	61	2400	26/38	N	N	Y	Y	N		N	
86	5343/11	3	M	Y	N	N	N	N	INGUINAL	1X0.5	MATTED	Y	UL	N	3	N	N	N	NL	P	NEG	74	2400	42/56	N	N	Y	Y	N		Y	2+
87	5365/11	45	F	Y	Y	N	N	N	CERVICAL	3X2	SINGLE	N	UL	N	2	N	N	N	PNEUMONITIS	P	NEG	68	2400	46/58	N	Y	Y	N	N		N	
88	5369/11	45	F	Y	Y	N	Y	N	CERVICAL	1.5X1	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	65	2400	36/52	N	Y	Y	N	N		N	
89	5371/11	28	F	Y	Y	N	N	N	CERVICAL	2X1	SINGLE	N	UL	N	2	N	N	N	PNEUMONITIS	INTM	NEG	62	2400	38/46	N	Y	Y	N	N		N	
90	5374/11	12	M	Y	N	N	Y	N	CERVICAL	3X2	MATTED	Y	UL	N	3	N	N	N	NL	P	NEG	69	2400	28/36	N	Y	Y	Y	Y	2+	Y	2+
91	5386/11	32	F	Y	Y	N	Y	N	AXILLARY	2X1	SINGLE	N	UL	N	2	N	N	N	BRONCHIECTASIS	P	NEG	72	2400	38/46	N	N	Y	Y	N		N	

S. No	CYTOLOGY NO	AGE	SEX	FEVER	WEIGHT LOSS	NIGHT SWEATS	COUGH	H/O ATT/ART	LYMPH NODE							SPLENO-MEGALY	HEPATO-MEGALY	OTHER CLINICAL FINDINGS	IMAGING	MANT-OUX	SPUTUM AFB	BLOOD INVESTIGATIONS				H&E			Z-N	GRADE	AO	GRADE
									SITE	SIZE (cm)	SINGLE/ MATTED	ABCESS	UL/BL	SINUS	STAGE (J&C)							LYM %	ABS. LYM.C	ESR (mm)	HIV	GIANT CELL	EPITHEL-IOID CELL	CASE-ATION				
92	5387/11	45	M	N	Y	N	N	N	CERVICAL	1X0.5	SINGLE	N	UL	N	2	N	N	N	NL	INTM	NEG	45	2400	24/32	N	N	Y	N	N		N	
93	5388/11	70	F	Y	Y	N	N	N	INGUINAL	2X2	MATTED	Y	BL	N	3	N	N	N	NL	P	NEG	62	2400	28/36	N	Y	Y	Y	N		N	
94	5399/11	17	M	Y	Y	N	N	N	CERVICAL	0.5X0.5	SINGLE	N	UL	N	2	N	N	N	NL	P	NEG	48	2400	24/32	N	N	Y	N	N		N	
95	5410/11	35	F	N	Y	N	N	N	CERVICAL	2X1	MATTED	N	BL	N	2	N	N	N	NL	N	NEG	62	2400	36/42	N	N	Y	N	N		N	
96	5419/11	13	F	N	N	N	Y	N	HYPOCHONDRIAL REGION, ANT. ABDOMINAL WALL	1.5X0.5		N	UL	N		N	Y	N	HILAR NODES+	P	NEG	68	2400	42/56	N	N	Y	Y	N		N	
97	5434/11	55	M	Y	Y	Y	Y	N	SUPRACLAVICULAR	1X1.5	SINGLE	Y	UL	N	3	N	N	N	PNEUMONITIS	P	NEG	48	2400	24/36	N	Y	Y	N	N		N	
98	5463/11	46	F	N	N	N	Y	N	CERVICAL	1X1	SINGLE	N	BL	N	1	N	N	N	NL	P	NEG	40	2400	28/42	N	N	N	N	N		N	
99	5471/11	42	M	Y	Y	Y	Y	H/OART	SUPRACLAVICULAR	3X2	MATTED	Y	UL	N	3	N	Y	N	CAVITY	N	NEG	16	520	32/42	P	N	Y	Y	N		N	
100	5483/11	8	F	Y	N	N	Y	N	CERVICAL	3X2	MATTED	Y	UL	N	3	N	N	N	NL	P	NEG	69	2400	28/36	N	Y	Y	Y	Y	2+	Y	2+

PROFORMA

PROFORMA

S.No

HOSPITAL ID NO-OP/IP

CYTOLOGY NO

NAME

AGE

SEX

ADDRESS

CHIEF COMPLAINTS

FEVER

WEIGHT LOSS

NIGHT SWEATS

COUGH / HEMOPTYSIS

LYMPHADENOPATHY

OTHERS

RELEVANT HISTORY
(occupation/contact/social/others)

EXAMINATION-GEN/LOCAL

LYMPH NODE EXAMINATION
SITE

SIZE

SINGLE/MATTED/CONSISTENCY

UL/BL

HEPATOMEGALY

SPLENOMEGALY

SINUS

FNAC

ADEQUACY

TYPE

POST-PROCEDURE
PATIENT STATUS

LAB-INVESTIGATIONS

BLOOD INVESTIGATIONS

CBC
ESR

ABSOLUTE LYMPHOCYTE COUNT

LYMPHOCYTE %

HIV

SPUTUM-AFB

IMAGING STUDIES

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BIBLIOGRAPHY

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ABSTRACT

SETTINGS:Tuberculosis is a major health problem in India and other developing countries. According to RNTCP reports one death occours each minute due to TB in India.Extra pulmonary TB largely manifests as lymh node enlargement. This study was undertaken to identify the presence of AFB in lymph node fine needle aspirates which were reported as granulomatous lesions by the routine H&E stain.AFB staining was done by Ziehl Neelsen and fluorescent stains and the results were compared to identify the most sensitive,rapid test. *METHODOLOGY* : Patients clinically suspected of TB with lymph node enlagement were included in the study 212 such cases referred to the cytology department at Stanley Medical College were included in the study.FNA was done as out patient procedur.Three smears were taken from each patient and stained for H&E, Ziehl Neelsen, and Auramine O fluorescent stain and the results compared to identify the sensitive test among ZN and Auramine fluorescent stain. Only the lesions reported as granulomatous were included in this study. *RESULTS* : 100 granulomatous lesions were taken for study and stained by ZN and Auramine O.AFB was identified in 11/100 cases.All the ZN positive cases were also positive for Auramine staining. In addition fluorescent stain identified AFB in 8 cases with a combined positivity of 19/100 cases. *CONCLUSION* : This study showed that the fluorescent stain detected more number of AFB cases than the conventional ZN stain and found to be more sensitive than ZN stain. Identification of bacilli was also easier since AFB could be identified under low power and it needed less number fields for screening. Thus Auraminr O fluorecent stain was found to be more sensitive than the conventional ZN stain in the identification of AFB in TB lymph node FNAC specimens and helped in arriving at a definitive cytological diagnosis.

KEY WORDS

FNAC, AFB, Ziehl Neelsen, auramine O fluorescent stain